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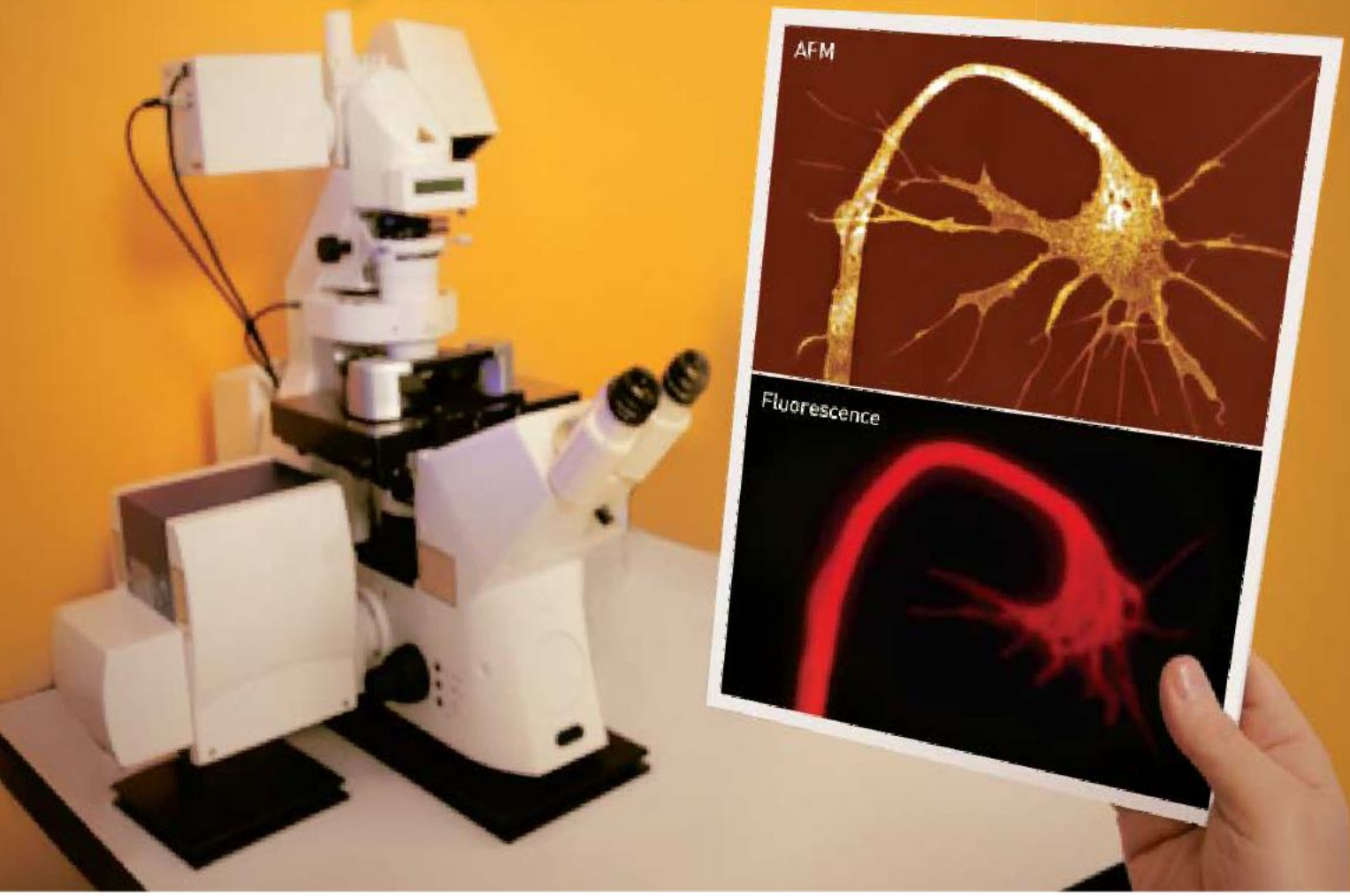

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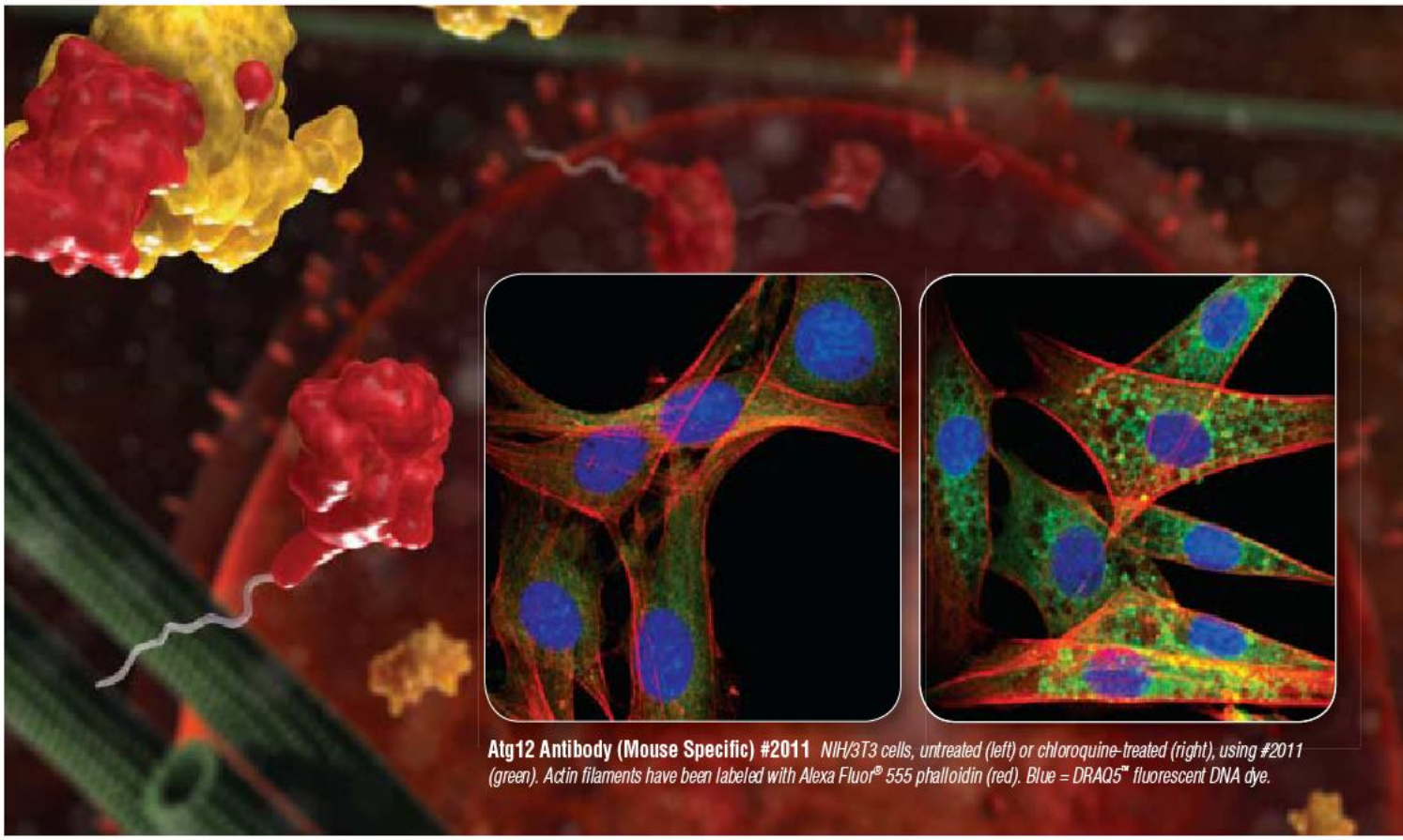

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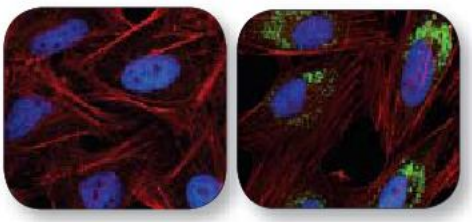

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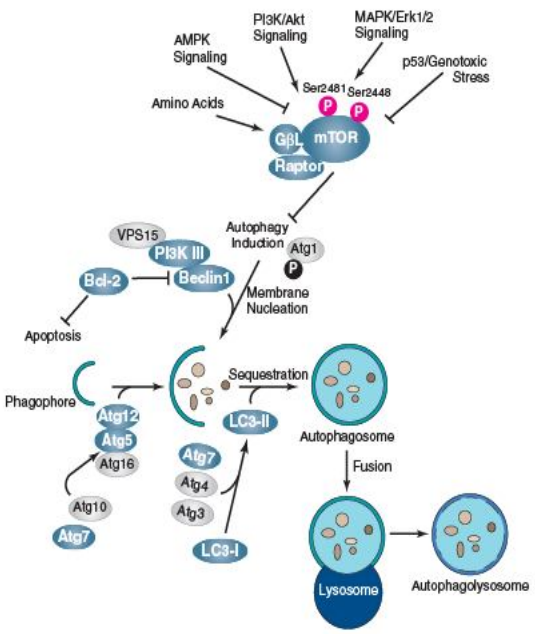

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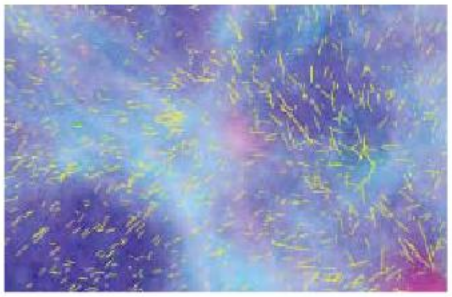

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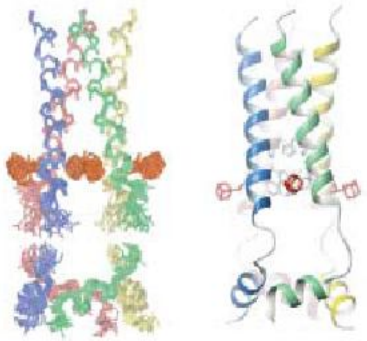

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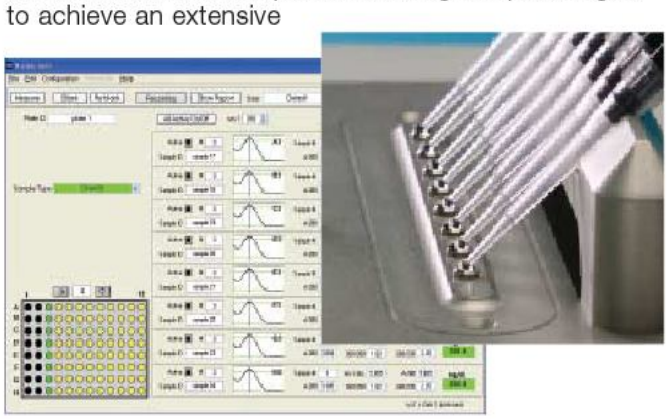

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THIS ISSUE

NATURAL-BORN KILLERS? Are violence, war and murder inevitable features of human life, governed by evolution as much as by social and historical factors? Dan Jones explores the complex relationship between morals and violence, and what evolution tells us about why humans kill. [News Feature p. **512**]

FOOD FOR THOUGHT The Correspondence columns this week comment on the merits and demerits of cognitive enhancement, prompted by the Commentary 'Professor's little helper', by Barbara Sahakian and Sharon Morein-Zamir (*Nature* 450, 1157–1159; 2007), and the continuing online Nature Forum thread 'Would you boost your brain power?' (http://tinyurl.com/2qpxff). [Correspondence p. **520**]

DISEASE LINKAGE Early genome-wide association studies had a reputation for turning up many false positives. But last year saw replicated studies identifying loci associated with type 2 diabetes, Crohn's disease and cardiovascular disease. Many more are on the way. Monya Baker takes the temperature of this hot field of research and asks how geneticists and clinicians will use the masses of data now set to flow. [News Feature p. **516**]

ENLIGHTENMENT SCIENCE The remarkable collections of the University History Museum in Pavia represent some of the key turning points in the history of science. Alison Abbott marvels at the pickled body parts, old physics instruments and architectural masterpieces within. [Books & Arts p. **526**]

RNA IN CHARGE The electron micrograph, below, of a mating pair of the ciliate *Oxytricha* (also called *Sterkiella*), which appeared on the Contents page in the

10 January issue, should be credited to Robert L. Hammersmith. As the accompanying paper reveals, nuclear development in this protozoan involves genome-wide DNA rearrangements directed by maternal RNA templates. [doi:10.1038/nature06452]

The idea that DNA base pairing could direct the crystallization of useful materials is a tempting one for nanotechnologists. Now — over ten years after it was first shown that DNA attached to nanoparticles can influence their assembly — two groups have put this concept into practice. Park *et al.* demonstrate that the DNA molecules attached to gold nanoparticles, and DNA molecules used to link them, can be selected to ensure that the nanoparticles self-assemble into either face-centred cubic or body-centred cubic crystals. The cover graphic, by Cole Krumbholz, is a close-up of the latter. [Letter p. **553**] Nykypanchuk *et al.* identify the requirements for DNA design and the crystallization conditions that allow the reversible formation of body-centred cubic crystals, with nanoparticles occupying just a few percent of a lattice volume. [Letter p. **549**] As discussed in News & Views [p. **528**], these developments might make it possible to create ordered and tunable 3D nanoscale architectures relevant for photonic and magnetic applications, biomedical sensing, and information or energy storage.

A glimpse of dark energy

Cosmologists can tell from observing distant supernovae that the Universe is undergoing a phase of accelerated expansion, but the physical cause remains a mystery. The favoured explanation requires huge amounts of invisible 'dark energy', distributed across the Universe, forcing expansion via gravitational repulsion. A new survey of redshift distortions of thousands of faint galaxies provides hints as to the nature of dark-energy induced cosmic acceleration. The distortion at a redshift of $z = 0.8$ is consistent with the standard cosmological-constant model with low matter density and flat geometry. The current error bars are too large to distinguish among alternative origins for the accelerated expansion, but the next generation of galactic surveys, more powerful and far-reaching than the current crop, should provide much tighter constraints on the properties of dark energy. [Letter p. **541**; News & Views p. **531**]

Sex determined by degrees

In mammals and birds, sex is determined by genotype, at fertilization. But many reptiles, hedge their bets, determining the sex of an individual by interaction with the environment, typically temperature. Thirty years ago, Eric Charnov and James Bull (*Nature* 266, 828–830; 1977) speculated that environmental sex determination will be favoured by selection if it could be shown that different temperature regimes maximized reproductive fitness for each sex. Until now it has not been confirmed, partly because of the difficulty of setting up a 'control' experiment in which the

DAN GARNETT

Sexual chemistry: the 'Jacky dragon' lizard proves a point.

'wrong' sex is produced at a given temperature. Hormone treatments have been used to overcome this difficulty, and Daniel Warner and Rick Shine now confirm, in a species of Australian lizard, that the Charnov/Bull model is correct. [Letter p. **566**; News & Views p. **527**]

Piezoelectrics made simple

Application of mechanical force to a piezoelectric material generates a voltage; conversely, apply a voltage and you get a force. This combination of properties has many applications, primarily in the generation of ultrasound. The largest electromechanical responses tend to occur in highly complex materials, and the desired properties tend to be maximum when associated with a 'morphotropic' phase transition — an abrupt structural change usually linked to changes in composition. Muhtar Ahart *et al.* show that a similar phase transition can occur in a simple, pure compound, under high pressure. The compound is the prototypical ferroelectric, lead titanate, and it produces an electromechanical response larger than any known. It may be possible to chemically tune these effects to ambient pressures, which would potentially reduce the costs

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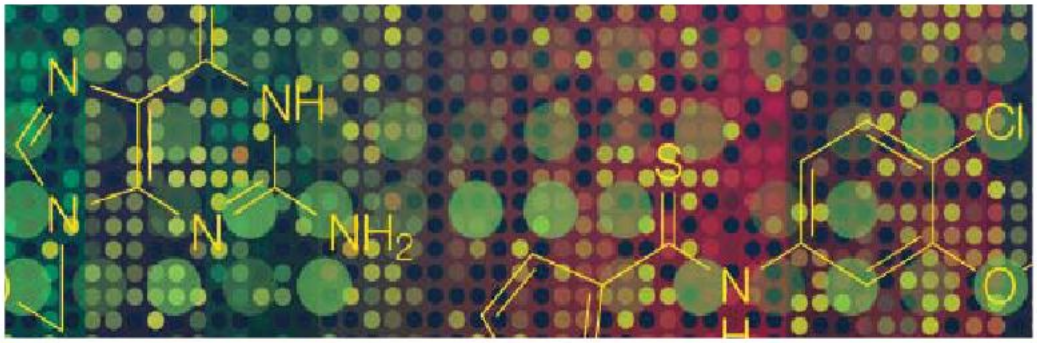

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and enhance the utility of high-performance piezoelectric materials. [Letter p. 545]

Expression patterns

For developmental biologists, *Drosophila* embryo segmentation is the standard model for the study of body pattern formation. It is a well characterized system, yet one area where information is sparse is transcriptional control — the mechanisms that establish complex expression patterns at precise times and locations. A new computational framework described in this issue goes a long way towards filling this gap. The algorithm takes as input the DNA binding specificities and expression levels of key transcription factors and predicts the expression level that any given *cis*-regulatory sequence will produce at every spatial position along the antero-posterior axis of the embryo. The algorithm is generally applicable, so may prove useful for many other protein–DNA interaction systems. [Article p. 535]

Atlantic hurricanes

The recent increase in Atlantic hurricane activity is widely believed to be due in part to rising sea surface temperatures, but to what extent is not known. Mark Saunders and Adam Lea quantify this contribution for storms forming in the tropical North Atlantic, Caribbean Sea and Gulf of Mexico using a statistical model based on two environmental variables — local sea surface temperature and an atmospheric wind field. They conclude that local sea surface warming was responsible for about 40 per cent of the increase in hurricane activity (relative to the 1950–2000 average) between 1996 and 2005. [Letter p. 557]

Danger in numbers

Two recent great earthquakes near the Kuril islands — the Pacific group often in the news because of a sovereignty dispute between Japan and Russia — dramatically demonstrate the process by which large subduction-zone earthquakes can influence the stresses and earthquake activity within the subducting oceanic plate itself. On 15 November 2006, a magnitude-8.3 event ruptured the shallow-dipping plate boundary where the Pacific plate descends beneath the central Kuril arc. Within minutes, intraplate extensional earthquakes occurred in the outer rise region seaward of the Kuril trench. Then on 13 January 2007, a magnitude-8.1 event ruptured a normal fault extending through the upper portion of the Pacific plate, producing one of the largest recorded shallow extensional earthquakes. [Letter p. 561]

Sleeping with the worms

Sleep research enters uncharted territory with the discovery of a sleep-like state in the nematode *Caenorhabditis elegans*. Sleep is vital in most animals but why this is so is largely unknown. The newly discovered quiescent behavioural state in *C. elegans*, called lethargus, displays many similarities with sleep as defined in mammals and, more recently, in *Drosophila*. Lethargus is regulated by a cyclic GMP signalling pathway, the first time this mechanism has been linked to sleep but probably not the last, since a similar pathway is conserved in *Drosophila*. [Letter p. 569]

The heart under stress

AMPK (AMP-activated protein kinase) is a master regulator of many biological processes and is a potential drug target for diseases as diverse as diabetes, cancer, atherosclerosis and ischaemic heart disease. In the ischaemic heart, AMPK protects tissues from injury and apoptosis by promoting glucose uptake. A new study shows that AMPK is activated by the inflammatory cytokine MIF (macrophage migration inhibitory factor), which is released by the heart under ischaemic stress. This throws new light on the nature of the cellular stress response, and highlights low MIF levels as a potential risk marker for patients with coronary artery disease. [Letter p. 578]

Linking ageing and cancer

The deacetylase SIRT1, the mammalian version of the Sir2 protein that plays a critical role in determining lifespan in yeast, has been implicated in various cellular functions and possibly in tumorigenesis and ageing. The factors regulating SIRT1 are not well known but two groups now report that the tumour suppressor DBC1 is a negative regulator of SIRT1. By inhibiting SIRT1, DBC1 promotes increased acetylation of the tumour suppressor p53 and p53-mediated apoptosis in human cells. [Letters pp. 583, 587]

Influenza changes channels

Until recently, the pH-gated proton channel of influenza A virus, M2, was effectively targeted by amantadine-based antivirals, but resistance to these drugs is now widespread. Two groups now present structural studies of M2 proton channel. Jason Schnell and James Chou determine the structure of a 38-residue segment of M2, in complex with rimantadine, by NMR spectroscopy. [Letter p. 591] Amanda Stouffer *et al.* determined the crystal structure of a 25-residue fragment of M2, with and without amantadine, using X-ray diffraction. [Letter p. 596] Strikingly, the resulting structures suggest two very different mechanisms by which the drug inhibits the channel. The proposed mechanisms are discussed by Christopher Miller in an accompanying News & Views article [p. 532]

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Abstractions

FIRST AUTHOR

In 1977, Eric Charnov and James Bull proposed a now widely accepted evolutionary model to explain why the gender of some species of lizard, turtle, crocodilian and fish is determined not by genetics, but by environmental factors such as temperature. Until now, however, no experimental evidence had confirmed an adaptive benefit — and thus evolutionary significance — of temperature-dependent sex determination (TSD). Daniel Warner, now a postdoc at Iowa State University in Ames, took on a risky PhD project and showed that developing at certain incubation temperatures increases the reproductive success of each sex (see page 566). Warner spoke to *Nature* about how his gamble paid off.

What hurdles had to be overcome to do this experiment?

For many years it was impossible to properly compare reproductive fitness in TSD species, because there was no way to produce both genders over a range of temperatures. A further complication was that TSD was known only in long-lived species. Recent work demonstrated that chemicals can interfere with hormone synthesis and allow us to override the temperature control of sex determination. And, in 2000, the jacky dragon, an Australian lizard with a 4–5-year lifespan, was discovered to have TSD.

Why take on such a risky project?

Raising 200 jacky dragon hatchlings in semi-natural field conditions over 3 years was a lot of work. I was worried something might go wrong, like feral cats eating my population. But I knew this was my best chance to test Charnov and Bull's hypothesis. My heart was in it, and I really wanted to give it a shot.

Were you surprised by your findings?

After the first major reproductive season, I assigned fathers to offspring using genetic analyses to measure the reproductive success of parents produced at different temperatures. I was shocked by how clearly the pattern reflected the theory's predictions. I was nervous that more data would swamp that pattern, but they actually reinforced it.

How might global warming affect TSD species?

We could see a major shift in sex ratios — which could affect viability, if not cause extinctions, in some populations.

Have Charnov or Bull contacted you?

Yes, they are both aware of this work, and I think they are pleased to see their theory supported. That's really exciting for me. These are the gurus in the field, and I look up to their work. ■

MAKING THE PAPER

Ronald Cohen

Computational theory reveals an unusual quality in a pure compound.

"If you look at something closely that is thought to be well understood, you often find something new and exciting," says Ronald Cohen, speaking of his latest work with lead titanate. Researchers thought that this simple compound could be "easily understood" as early as the 1950s. But Cohen, of the Carnegie Institution of Washington Geophysical Laboratory, and his colleague Zhigang Wu, revealed an unexpected property, using a new theory developed by computational physicists to calculate the properties of piezoelectrics — substances that convert electrical energy to mechanical energy and vice versa.

When they applied the theory in the context of extreme pressures, Wu and Cohen discovered that lead titanate would undergo a set of unexpected phase transitions never before seen in a pure compound but usually associated with more complex, and commonly used, piezoelectric materials. These structural changes — and the electromechanical responses that accompany them — render such materials useful for a range of applications, from medical ultrasound to sonar.

Wu and Cohen's results suggested that simple compounds could be developed for similar applications. The computed piezoelectric properties of pure lead titanate under pressure were greater than those of any known material.

After celebrating their theoretical discovery, the Geophysical Lab team decided to put the theory to experimental test. The first thing the researchers had to do was create the conditions in which lead titanate was expected to take on the properties in question and find ways to measure these properties. "To do both together is extremely difficult," says Cohen. The team first tried using Raman spectroscopy to measure atomic vibrations at high pressures and low temperature (10 kelvin). "Those experiments showed interesting behaviour for the compound, but we didn't know exactly what it meant," says Cohen.

They turned to X-ray diffraction, using the Advanced Photon Sources at the Argonne National Laboratory in Illinois, again under high pressures and at cryogenic temperatures. To obtain useful data they had to perform high-resolution diffraction experiments, which take hours rather than minutes or seconds. "It's difficult to get that amount of synchrotron time, and it's also hard to maintain the required pressure and temperature for so long," says Cohen.

But the authors' perseverance paid off. They eventually obtained a high-resolution image of lead titanate undergoing a morphological metamorphosis that Cohen describes as "akin to making a cube switch from sitting on a face to balancing on a corner" (see page 545). This type of structural change is associated with a large electromechanical response.

The team decided to take the work a step further and used this information to design a compound that would have similar electromechanical properties to lead titanate but at ambient temperature and pressure. The next challenge is to actually make this material and confirm its properties.

"If, as I hope, we succeed in developing useful new materials by this route, we will have shown the utility of a materials-by-design approach for the next generation of technological materials," Cohen says. ■

FROM THE BLOGOSPHERE

The journal *Nature Chemistry* will not appear until early 2009, but its chief editor, Stuart Cantrill, is already planning its content ... and writing about it. Cantrill writes on the Sceptical Chymist, the NPG blog that discusses research and events of interest to the chemical community, that the name is the only part of the journal that exists so far — but that things will quickly change. In his series of 'Journal journeys', Cantrill is keeping a diary of how *Nature Chemistry* is taking shape. Readers can follow the journeys at http://blogs.nature.com/thescepticalchymist/features/journal_journeys/.

The first entry, posted on 21 January, is called 'Day −11', because Cantrill officially begins his new role on 1 February. Until then, he will be handling manuscripts submitted to *Nature Nanotechnology*, where he is currently senior editor.

His first task for *Nature Chemistry* will begin on 31 January, the closing date for applications for associate editor positions. He will be looking through them all, but seeking out those that contain no spelling mistakes and include all the requested information and documents. ■

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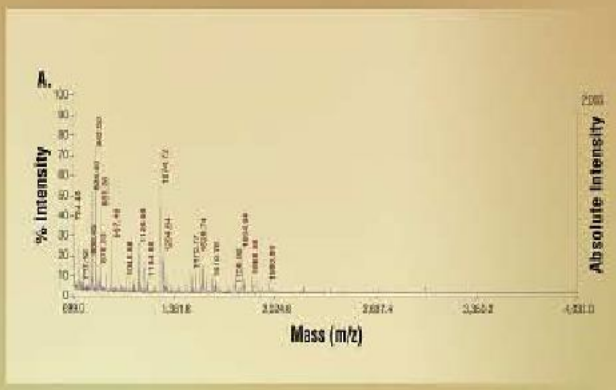
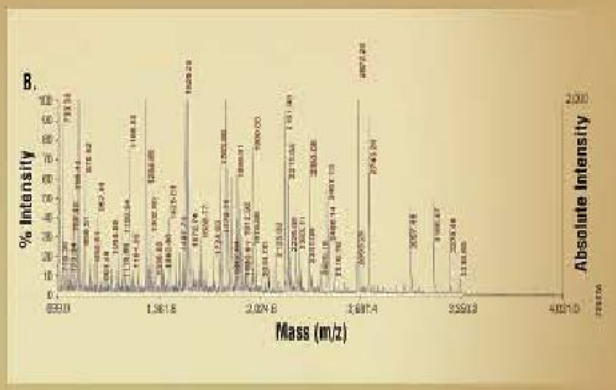


Panel A: Current standard: in-gel digestion of 47kDa protein band from mouse cytoplasmic protein extract using Trypsin Gold.



Panel B: More complete in-gel digestion of 47kDa protein band from mouse cytoplasmic protein extract using Trypsin Gold with ProteasMAX™ Surfactant.

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Vol 451 | Issue no. 7178 | 31 January 2008

Towards falling emissions

Although Europe's new energy plans may be too prescriptive on the means of achieving the goals, they offer the world an encouraging way forward.

The problem with action on climate change is that politically plausible programmes have a tendency to sound too small for the size of the challenge, whereas plans that match the challenge in their scope tend not to sound politically plausible. It is possible, though, that the plans drawn up by the European Union (EU) for a directive on action to reduce the carbon dioxide emissions of the largest economy on Earth, announced on 23 January, have managed to hit the sweet spot in between.

The idea of a '2020' policy — 20% less carbon emitted by 2020, with 20% of all energy coming from renewable sources by the same date — was first agreed by members of the EU last March. The latest announcement represents the view of how to do this, taken by the European Commission, the EU's executive (see page 504).

Commendably, the proposal sets a clear medium-term goal. There is a tendency, when faced by the daunting challenge of re-engineering the planet's energy infrastructure, to set goals that are ever more ambitious but ever farther off in the future. The commission's policy avoids that, and sends a clear signal to industry about the target to aim for and the means by which Europe will get there: an expansion and tightening of its carbon-trading scheme.

Another welcome aspect is that the proposals are seen by their framers as a minimum. If other major economies sign up for carbon cuts, the EU says it will be willing to step up its own efforts. This is not excessive modesty of goals, but is in part a response to the risk of carbon emissions simply being moved offshore; if European requirements are draconian compared with those elsewhere, then emissions-intensive industries will move away. This would not only mean that jobs and investment would be lost to Europe; it would also, in all likelihood, entrench a laissez-faire attitude to emissions in the economies where those industries ended up.

Fair trade

Similar considerations are seen in the commission's decision not necessarily to do to energy-intensive industries what it is doing to electricity utilities — forcing them to pay for their carbon credits from 2013 on. They are also at play in a willingness to talk, so far in only vague terms, about ways in which these issues might be brought into global trade agreements, perhaps with tariffs on imports from countries that do not put a cost on carbon emissions. These fears of 'carbon leakage' may be overblown, and their popularity with the industries that benefit from such special pleading and traditionally protectionist lobbies definitely calls them into question. But the idea that one can realign the planet's industrial base with no impact on its international trade regime does seem, on the face of it, unlikely. The precedent for giving environmental issues standing at the World Trade Organization has, after all, already been set (albeit on the rather less contentious issue of turtle-friendly nets for prawn fishermen).

A trade war would be a steep and unnecessary price to pay. Far better for other major economies to follow Europe's lead and set in place similar statutory reductions in emissions. They should not be so keen, though, to emulate other aspects of the plan. Adding a second sonorous '20' to a plan for 2020, for example, is hardly a justification for requiring renewable sources to make up 20% of the total energy mix. Renewable energy is only one low-carbon option: nuclear energy and fossil-fuel energy with carbon capture and storage also need to be pursued. Renewables have their advantages, but a hard and fast target hardly seems the government intervention that will necessarily best foster the revolution in renewable-energy technologies touted by various visionaries. Such monolithic planning sits poorly with the eclectic entrepreneurialism that is the hallmark of much of the best of the clean-energy sector.

"The European Union's plan offers a way forward that is neither fantastical nor laggardly."

Fuel for thought

The plan to mandate 10% use of biofuels in the transportation sector raises similar concerns. Some biofuels are a good thing and some aren't — those derived from crops that are also a source of food are in general best avoided. It is possible that 'second- and third-generation' biofuels, made in new ways from the sorts of biomass best suited to the job, will be a better deal. This area, like many others in the energy sector, will benefit from more research and development funding, both from governments and the private sector. But to assume that the results will be well suited for 10% of the market just because you say so is foolish.

This over-emphasis on fuel-mix targets reflects a broader problem with the discourse of climate change: it tends to be positioned between public measures on the supply side and individual attempts at conservation on the demand side. But public measures can do a lot for the demand side, too — as indeed the EU is attempting elsewhere — and supply-and-demand measures need to be considered together. Increasing energy efficiency — in the home, in transport, in industry — is an area where governments have a lot to offer. This was appropriately emphasized by Japan's prime minister Yasuo Fukuda at the World Economic Forum in Davos, Switzerland, last week, as a key issue for the G8 summit in Hokkaido in July.

It is important, in the horse-trading that will now ensue between member states as the commission's plans are turned into policy, that their core commitments are not weakened by special pleading. The final policies must be fully verifiable and enforceable. But the new plan offers a way forward that is neither fantastical nor laggardly. It says that if the will is maintained, a major economy can make the crucial historic inflection from a world in which emissions always rise to one in which they fall. They may need to fall faster; but that will be much easier to achieve once they are set to fall in the first place. This is a serious step in that direction, and should be welcomed. ■

Secret treasure-troves restored

Reflecting on the endeavours of scientists past can provide both inspiration and pleasure.

The face of science is always turned to the future — and that has been the downfall of many a historic scientific collection outside of the mainstream museums.

Take the eighteenth- and nineteenth-century natural-history collection begun in the 1770s by Lazzaro Spallanzani at the University of Pavia in northern Italy. In the 1930s, the half-million stuffed specimens — from giant turtles to gibbons — were cleared out to make room for a new faculty of law. The collection had become a slight embarrassment, a woefully old-fashioned way of doing science. After sojourning at a nearby palazzo, it was carelessly crammed into the attics of the local Visconti castle for storage. But even the finest address can host insects and microbes, and over the years the collection began to rot.

Similar fates befell other collections around Europe. At best, individual items would be given as amusing gifts to visiting scientists, at worst, the whole lot would be thrown away. Research interests had moved on, teaching methods modernized and, when the student numbers mushroomed after the 1960s, space was needed by new faculty members.

But in recent years a fresh awareness has developed about such relics, stirring first in Italy, whose scientific history, from the Renaissance to the start of the twentieth century, is arguably the most important on the continent. In 1991, the Italian association of university rectors set up a committee specifically to ensure that collections in universities were catalogued and cared for. In 2004, the Italian government was persuaded to amend its law on cultural heritage to include the protection of scientific objects, and the next year the Council of Europe passed a like-minded resolution addressing universities across the continent.

"In recent years a fresh awareness has developed about historic scientific collections."

Germany was alerted to what is hidden in the forgotten corners of its old universities when those in eastern Germany were required to make inventories at the time of reunification. A project to comprehensively digitize the collections in all the nation's universities is now under way.

Even so, there is little money across the continent for restoration. Back in Pavia, the university cannot find the resources to speed up the painstaking rescue of some of Spallanzani's specimens, and many will be lost forever. On the other hand, several of its other collections have survived well — and they form the basis of the first in *Nature*'s new monthly series paying homage to relatively unknown collections and other scientific monuments off the well-beaten museum track (see page 526). The series will, we hope, inspire a greater interest in where scientists have come from, as well as encouraging those on the conference circuit with a few hours to spare to visit them. Delight is guaranteed. ■

A quantum of solace

As the US writers' strike rolls on, now is the time for scientists to extend the hand of friendship.

In the universe inhabited by James Bond, science manifests itself in two ways: as nifty gadgets for the good guys and as new ways to destroy or conquer the world for the bad guys. Can we expect this to change in the next instalment, the intriguingly titled *Quantum of Solace*? Probably not. The title is taken from an Ian Fleming short story about what is required for love, as recounted at a dinner party, not what is needed for a Bose–Einstein condensate as measured in a physics lab.

No, the real subjects of the 007 series are always charisma and violence; and violence, it seems, is the preoccupation of a globally dark Zeitgeist. The fine films nominated for Oscars this year mostly trade in crime, bloodshed and war. Science has useful things to say about our relationship with violence (see page 512), but it is the science that enhances violence, rather than seeks to understand it, that interests filmmakers. Why is that masked man doing all these impossible things? Radioactivity! Aliens! Genetic-modification experiments gone horribly awry!

Naturally there are exceptions. This year's Sundance Film Festival was awash with earnest science documentaries, all children of *An Inconvenient Truth*. A forthcoming Meryl Streep picture, *Dark Matter*, attempts with mixed success to dramatize the life of hardworking Chinese graduate students in cosmology — although it does feature bloodshed too. Russell Crowe has twice been nominated for an Oscar for playing real-life scientists in serious films — mathematician John Nash in *A Beautiful Mind* and research chemist Jeffrey Wigand in *The Insider*. But he won his Academy Award for *Gladiator*.

"It is the science that enhances violence, rather than seeks to understand it, that interests filmmakers."

Scientists often complain that they can never change the way that science is portrayed in films, which seems as if the screenplays are written on a planet with different laws of physics. But, to quote an earlier Bond film, never say never. Indeed, today is a propitious time for such intervention. The Writers Guild of America has been on strike since 5 November. Its members want a better deal in relation to online and other relatively new distribution channels. And boredom among these picketing scribes may well be at an all-time high — a recent update on the strike from *The New York Times* was headlined 'For Strikers, the Agony of Spare Time'.

What better moment to saunter down to your local picket line, gather up a couple of film and television writers, and introduce them to the fascinations of the scientific life? Buying them a round might not hurt either; some of them have taken a serious financial hit. ■

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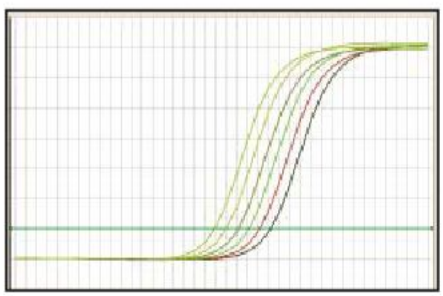

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RESEARCH HIGHLIGHTS

Tree-ring tales

Geology 36, 99–102 (2008)

Tree rings from the 'fossil forest' of Axel Heiberg Island in the far north of Canada are shedding light on the polar seasons of around 45 million years ago.

Hope Jahren, of Johns Hopkins University in Baltimore, Maryland, and Leonel Sternberg of the University of Miami in Coral Gables, Florida, studied carbon, oxygen and hydrogen isotopes within the well-preserved rings. They found unprecedented detail of month-to-month changes in the environment, such as a sharp increase in humidity during the growing season.

It is a rare look at the seasonal details of how forests grew in the unusually warm polar environment of the Eocene period.

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PHYSIOLOGY

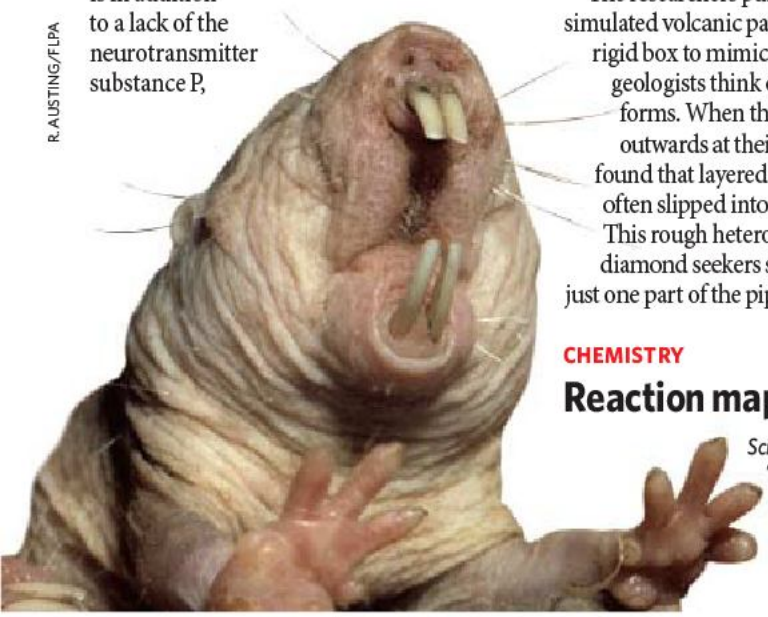
Acid test

PLoS Biol. 6, e13 (2008)

African naked mole-rats (*Heterocephalus glaber*, pictured below) do not feel pain from acid or capsaicin, the compound that makes chilli peppers hot; nor does their skin become more sensitive to temperature when it is inflamed, according to new work. On the other hand, they hate being pinched.

Thomas Park of the University of Illinois, Chicago, Gary Lewin of the Max-Delbruck Center for Molecular Medicine in Berlin and their colleagues explained the mole-rat's selective pain responses by comparing them with laboratory mice. The team uncovered unusual connections between the mole-rat's capsaicin-sensitive receptors and nerve cells called deep dorsal horn neurons. This is in addition to a lack of the neurotransmitter substance P, which the researchers had reported earlier.

Inducing the synthesis of substance P in mole-rats' hind legs gave the animals the ability to sense the burning of chilli applied to their hind feet, shown by the mole rats fretfully licking their feet.



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GEOLOGY

Pipe dream find

J. Volcanol. Geotherm. Res. doi:10.1016/j.jvolgeores.2007.12.034 (2008)

Diamonds are found mostly in kimberlite pipes —vertical, often cone-shaped columns of rock formed by eruptions within Earth's mantle. During an eruption, churning by volcanic gases is likely to keep diamonds from settling in certain parts of the pipes, according to models made by Thomas Gernon and his colleagues at the University of Bristol, UK.

The researchers pumped gas through simulated volcanic particles confined in a rigid box to mimic the violent mixing that geologists think occurs when kimberlite forms. When the box walls were angled outwards at their top end, the team found that layered regions on the periphery often slipped into the churning core. This rough heterogeneity suggests that diamond seekers shouldn't limit mining to just one part of the pipe, Gernon says.

CHEMISTRY

Reaction mapping

Science 319, 442–445 (2008)

The intricacies of the reactions at the interface of a gas and a solid catalyst have been probed with a technique that is normally the preserve of clinicians — magnetic resonance imaging (MRI).

Louis-S. Bouchard at the University of California in Berkeley and his colleagues used MRI to follow parahydrogen — a version of hydrogen in which the nuclear spins of two linked atoms go in opposite directions, cancelling each other out magnetically — as it passed over a solid rhodium catalyst.

During the reaction under scrutiny — hydrogenation of propylene to propane — the parahydrogen splits up, and each half suddenly has its own magnetic charge, which is picked up by the MRI.

The ability to follow the reaction in a microreactor will help engineers and chemists design better lab-on-a-chip devices, which are increasingly used in pharmaceutical synthesis and industrial catalytic reactions, the authors say.

PRIMATOLOGY

Death by research

Curr. Biol. doi:10.1016/j.cub.2008.01.012 (2008)

For the chimpanzees of Côte d'Ivoire's Taï research project, humans are a blessing and a curse. Researchers protect the chimps from poachers, but can also make them sick, say Fabian Leendertz from the Robert Koch Institute in Berlin and his co-workers. They are the first to document transmission of viruses from humans to wild chimps.

After three outbreaks of respiratory disease in the Taï chimps, tissue samples from seven chimps that had died revealed that they had two common human paramyxoviruses. These two viruses often cause respiratory infections in humans, and can kill human

infants. The researchers say that many fatal transmissions from researchers and tourists to wild apes have probably already occurred, and call for strict hygiene, including face masks, when humans interact with apes.

MINERALOGY

Directional diamond

Earth Planet Sci. Lett. doi: 10.1016/j.epsl.2007.11.052 (2007)

The mineral pyrrhotite, commonly found inside diamonds, acts as an ancient compass needle, recording where magnetic North was when the diamond formed.

It is not the only mineral that does so. Earth's magnetic field has oscillated between North and South throughout history and a few magnetic minerals, such as magnetite, are well known to record accurately the field's direction when the mineral formed. These minerals provide a record for geologists interpreting the positions of the continents through time. But according to Bradford Clement at Florida International University in Miami and his team, pyrrhotite could prove extremely useful to researchers studying the Cretaceous period, when diamonds were formed, because quality magnetic minerals from that period have been consistently hard to find.

DEVELOPMENTAL BIOLOGY

Batmouse

Genes Dev. **22**, 141–151 (2008)

Researchers have generated mice with longer forelimbs by inserting into their genomes a piece of DNA that regulates wing development in the short-tailed fruit bat, *Carollia perspicillata*. The result highlights the importance of regulatory DNA sequences in the evolution of diverse limb shapes.

The DNA fragment contained a sequence that enhances the expression of a gene called *Prx1*. *Prx1* governs limb development in mice and wing development in bats (pictured above), but when, where and how much it is expressed differs between the two animals.

Richard Behringer of the University of Texas at Houston and his co-workers switched a mouse *Prx1* regulatory sequence with that of a bat. Forelimbs in developing mouse embryos that carried the bat sequence were 6% longer than normal, but retained the shape of a normal mouse limb.

SUPRAMOLECULAR CHEMISTRY

Embrace the base

J. Am. Chem. Soc. **130**, 818–820 (2008)

DNA synthesis, the basic process of biological replication, has been followed one step at a time using a new molecular device created by Reza Ghadiri and his team at the Scripps Research Institute in La Jolla, California.

The enzyme DNA polymerase adds new bases one by one to a 'primer' DNA strand growing on a template strand that has a complementary sequence. To monitor this process, the researchers attached a polymer tether to one end of the template strand and threaded this through the narrow neck of the pore-forming protein α-haemolysin, which is inserted in a lipid membrane. With an end cap that prevents unthreading, the composite strand can be pulled into the neck of the pore and released again using an electric field. When lodged in the neck, the DNA affects the flow of ions through the channel, and the resultant change in ion current alters each time the primer strand is elongated by the addition of a new base.

BIOPHYSICS

Come together

J. Biol. Chem. doi:10.1074/jbc.M708898200 (2008)

When cells run short of calcium, they import more through 'store-operated' channels, which are ancient, ubiquitous and essential to many cell functions. Christoph Romanin at the University of Linz in Austria and his team have revealed details of how two proteins — ORAI1, which sits in the cell membrane, and STIM1 — come together to allow this import.

The authors used a technique called fluorescence resonance energy transfer microscopy, in which the energy needed to emit light passes from one molecular label to another only if they are within a few nanometres of each other. This showed that STIM1 can induce ORAI1's calcium-passing pore to open even when the two proteins do not form lasting clusters. The interaction is thought to involve a 'coiled-coil' domain — a structure that sticks out of the membrane into the body of the cell — on ORAI1.

JOURNAL CLUB

Nicholas Katsanis
Johns Hopkins University, Baltimore, Maryland

A geneticist wonders what it takes to prove causality.

In the post-genomic era, we are increasingly confronted by a torrent of variation data, originating from gene sequence, copy number and methylation patterns. To complicate matters further, I anticipate that a notable fraction of variation among individuals will be found to be relatively rare events. This would severely hamper our ability to implement statistical methods to associate variants with disease pathogenesis.

A recent paper by Carpten *et al.* (*Nature* **448**, 439–445; 2007) highlights just how difficult solving this problem can be. The authors found a somatic missense mutation in *AKT1* in a small number (2–6%) of breast, colon and ovarian cancers, and expended considerable effort establishing its link to tumour development. Experiments included solving the AKT1 protein's crystal structure; calculating the predicted effect of the missense change on the protein's conformation and binding abilities; gauging phosphorylation rates of the protein; identifying cellular localization; measuring transformational competency of the mutant versus wild-type allele; and checking the mutant protein's ability to induce cancer in a mouse model.

In light of recent efforts to understand the total mutational load in cancer (for some examples see F. Dahl *et al. Proc. Natl Acad. Sci. USA* **104**, 9387–9392; 2007; C. Greenman *et al. Nature* **446**, 153–158, 2007; T. Sjöblom *et al. Science* **314**, 268–274; 2006), these data are both exciting and sobering, because the idea of performing such an exhaustive analysis on a large allelic series is not tenable. The challenge, therefore, is to solve this problem by developing functional assays that are physiologically relevant; amenable to at least medium throughput; and applicable to a range of mechanistic questions (not just neoplasia, for example).

Discuss these papers at **http://blogs.nature.com/nature/journalclub**

NEWS

Europe spells out action plan for emissions targets

Power stations in Europe will have to pay for permits to release carbon dioxide as soon as 2013, the European Commission announced last week. But although heavy industries will be included in the European Union's emissions trading scheme (ETS), the commission stopped short of making sectors such as steel and paper pay for their permits.

Commission president José Manuel Barroso proposed a package of market-based and regulatory measures to the European Parliament and Council of Ministers in Brussels on 23 January. He says that the package will bolster the European Union's leading role in tackling climate change and secure its future energy supply. If approved, the proposals could become law in the European Union's 27 member states as soon as 2009.

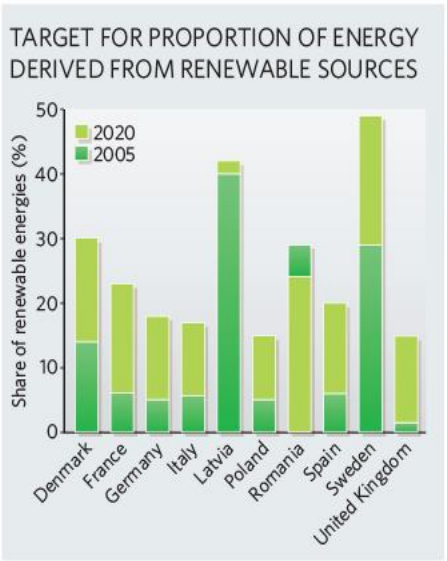
"Finally, we move from targets to tools."

"This is a strong proposal — stronger than I would have expected — and a very positive step forward in terms of climate protection," says Michael Grubb, a specialist on climate-change policy at Imperial College London.

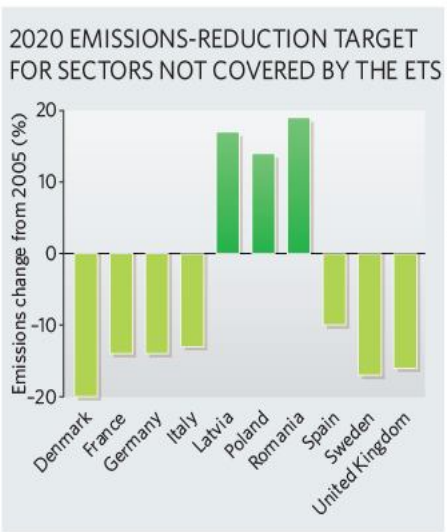
The details of the plan for how to cut greenhouse-gas emissions across the European Union by at least 20% by 2020 were hotly contested in Brussels. At the plan's core is an amendment of the ETS, first introduced in 2005, which has so far failed to facilitate a shift to low-carbon technologies. To give the ETS some teeth, the commission proposes a sweeping reform: most importantly, that power plants and energy-intensive industries will no longer receive a generous allocation of emissions 'allowances' free of charge. Instead, they will have to buy all allowances at auctions organized by the member states. Heavy industries that face strong international competition, including steel, aluminium, concrete and paper, will still get free allowances — although a consultation in 2011 will review this exemption.

Under current rules, which will remain valid until 2012, the 10,000 or so facilities that participate in the ETS get free emissions allowances from national packages that governments negotiate for every year. If a plant emits in excess of its free allocation, though, it must buy additional allowances on the emissions market. An allowance to emit one extra tonne of CO_2 in the 2008–12 trade period last week cost around €21 (US$31) on Europe's carbon-future markets.

Because national governments have in the past tended to over-allocate free allowances, the commission suggests doing away with national allocation plans and introducing instead a single cap throughout the European Union that is based on historic emissions and expected trends. "Centralizing the allocation of emissions rights may prove the most contentious point in the proposals," Grubb says. "But I think there is limited [room for] manoeuvre for the member states to unpick these proposals — they are in a weak position, given how they have behaved in the past."



SOURCE: EUROPEAN COMMISSION



SOURCE: EUROPEAN COMMISSION

In addition, the overall number of emissions allowances will be substantially reduced during the third trading phase, which starts in 2013. The commission suggests putting 1,974 million tonnes of tradable emissions allowances on the market in 2013, compared with 2,080 million tonnes today, and then reducing this yearly to 1,720 million tonnes by 2020. This would mean that emissions by all participants in the ETS — which account for around half of the European Union's total CO_2 emissions — would be reduced by 21% relative to 2005. The price of emissions allowances is expected to rise as they become scarcer, making it increasingly profitable for plant owners to switch to cleaner fuels, or to invest in clean-energy technologies such as carbon capture and storage.

The commission's proposals got cross-party support in the European Parliament last week, even though the cost of the package is estimated at €90 billion or 0.6% of gross

TRAVELPIX/GETTY

Power plants in Europe will no longer receive free allowances for their greenhouse-gas emissions.

domestic product in 2020. "There is a cost, but it is manageable," Barroso told parliament. "Implementing the package will cost €3 per week per EU citizen. Inaction will cost them €50–60 per week." The commission estimates that "by 2020 a household's overall energy bill would rise by an average of €150 per year".

"Finally, we move from targets to tools," says Lena Ek, a Swedish member of the Liberal Democrat party. British parliamentarian Graham Watson called the package "the most important act of the Barroso commission so far".

Auctioning emissions allowances could generate state revenues of up to €50 billion per year. At present, roughly the same sum is tantamount to a windfall profit for the power industry, which has passed on to consumers the 'costs' of emissions trading. The commission has not specified how member states should use the extra money, but does suggest that climate and energy research should benefit.

"Energy research and development in the European Union is shamefully low," says Ottmar Edenhofer, an economist at the Potsdam Institute of Climate Impact Research in Germany. "The proposed scheme will increase pressure on industry to develop low-emissions technologies. The revenues from auctioning emissions rights should be channelled into research and innovation."

The emissions market is volatile and has reacted nervously to political interference in the past, but experts say that the scheme will benefit from the amendments. "Industry, banks and investors need planning reliability," says Stefan Kleeberg, a carbon-market analyst with the 3C group near Frankfurt, Germany. "Knowing the post-2012 trading rules early will stabilize the market and will ultimately make it more efficient." Realistically, a price of €24–30 per tonne of CO_2 can be expected at auctions, he says.

Other proposals include sectors not covered by the ETS, such as transport, agriculture and buildings, which will on average have to reduce emissions by merely 10% of 2005 levels by 2020. Wealthier member states will need to cut more emissions than poorer countries.

In addition, the overall share of renewable energy in the European Union's final energy consumption is to increase from 8.5% to 20%, with specific targets for each member state. Countries that fail to deliver the legally binding target might face financial penalties.

The commission also calls for a 10% biofuel component in vehicle fuel by 2020. As excessive production of raw materials for biofuels has sparked serious environmental concerns, it will outline stringent sustainability criteria for their use. ■

Quirin Schiermeier

See Editorial, page 499.

Canada abolishes its national science adviser

The Canadian government is closing its Office of the National Science Advisor at the end of March, after just four years of service. The top-level science and technology adviser post will also be abolished in the move; it was already sidelined in 2006 when the new conservative government reassigned the adviser's responsibilities from reporting to the prime minister to reporting to the industry minister.

"I'm dismayed that the office is disappearing after four years and that it hasn't become a permanent fixture in science and technology in Canada," says Arthur Carty, who has held the post since its inception. Carty decided to retire from public office when he was told that the government was discontinuing the position.

Science adviser Arthur Carty has retired.

Industry Canada said that the decision to phase out the office followed the establishment of the Science, Technology and Innovation Council (STIC) in June 2007. "The STIC will function as a single committee, providing the government with independent and integrated advice on science and technology," it said. It consists of a chair, Howard Alper, a chemistry professor at the University of Ottawa who is former president of the Royal Society of Canada, and 17 members, including university leaders, scientists, industry executives and government ministers.

Canadian scientists are undecided as to whether the council can replace the role and function of a national science adviser. "I don't think what they have now in its place is any more likely to succeed than anything else," says David Anderson, director of the Guelph Institute for the Environment in Ontario and former federal environment minister.

"The jury is still out," says Elizabeth Dowdeswell, chair of the scientific advisory committee of the Council of Canadian Academies in Ottawa, Ontario.

Some scientists have criticized the move as evidence of the government's lack of interest in science and understanding of how it is done. Anderson says that Carty must have had a hard time giving science advice while the administration was trying to discredit the science of climate change.

Carty is a British-born organometallic chemist who ran the National Research Council Canada from 1994 to 2004. When Prime Minister Paul Martin revived the science adviser post to harness Canada's science potential and offer insight on international science issues, he appointed Carty. It was the first such post in Canada for 30 years (see *Nature* 427, 91; 2004). The office's small budget and vague mandate soured its chance of success from the start. Its finances, including salaries, hovered around Can$1 million (US$1 million), and the office didn't secure any permanent staff to help Carty until its third year.

During his tenure, Carty spearheaded the 2005 creation of the Council of Canadian Academies, like the US National Academy of Sciences. The council provides independent assessments of the science underlying key issues, but does not make recommendations to the government. Carty helped to establish Canada as a leader in the International Polar Year, ensuring that it provided $150 million in funding. He also represented Canada at the Carnegie Group's meetings of science ministers and advisers.

But some work, including a national consultation on how major science initiatives should seek funding, "never saw the light of day", Carty told *Nature*. "I don't really think the government has understood the role that a national science adviser — or that office — can play."

The news comes shortly after the government sacked Linda Keen, the president of the Canadian Nuclear Safety Commission, the country's independent nuclear watchdog. Critics have said that Keen was fired for "doing her job". The move suggests that the independence of advisers and committees is on shaky ground, says Anderson. ■

Hannah Hoag

ON THE RECORD

"To do this would be a radical violation of human dignity."

The Roman Catholic bishops of England and Wales claim that pending legislation will allow researchers to make half-human-half-animal embryos using the egg of a woman and sperm of an animal. "A radical violation of the truth," was scientists' response to the bishops' statement.

SCORE CARD

▲ **Animal health** Pedometers are being fitted to British dairy cows to monitor their health — they don't walk as far when they are sick.

▼ **Animal mental health** German zoologist Peter Arras has called Berlin Zoo's celebrity polar bear Knut a "psychopath" and says that he won't be able to mate.

ZOO NEWS

Poor Polly

A bicycle that was abandoned by a man trying to enter Belarus illegally last week turned out to be harbouring 277 parrots, stuffed 40 to 50 to a cage. Authorities will offer the birds to pet shops.

M. DURHAM/NATUREPL.COM

OVERHYPED

Bigfoot on Mars?

A NASA image showing a humanoid on Mars was widely circulated by the media last week. Sidelines is surprised that they had the time, what with the hundreds of sightings of Elvis here on Earth that need following up...

Sources: Catholic Bishops' Conference of England and Wales, The Times, The Independent, Reuters, NASA

SIDELINES

Cash for Russian nuclear scientists criticized

A post-cold-war US programme that pays nuclear weapons scientists from the former Soviet Union to prevent them working for 'rogue' states has come under fire in Congress, after a governmental investigative report questioned its usefulness.

The Initiatives for Proliferation Prevention (IPP) programme run by the Department of Energy (DOE) pays nuclear scientists in Russia, Ukraine, Libya, Kazakhstan, Armenia, Uzbekistan and Iraq to work on non-proliferation technologies with potential commercial applications. It was established in 1994 after the fall of the Soviet Union, with the goal of keeping destitute scientists in jobs and discouraging them from working with states that pursue nuclear programmes outside international agreements, such as Pakistan. But fewer than half the scientists currently being paid by the IPP have any weapons expertise, and programme money has been used to recruit new weapons scientists too young to have been part of the Soviet weapons complex, according to testimony to a Congressional committee last week by Robert Robinson, a managing director at the US Government Accountability Office (GAO). According to a GAO report published last month, the DOE has no exit strategy for the programme — no criteria to 'graduate' institutes and individuals from the programme once they cease being proliferation risks.

AP

Ex-Soviet soldiers destroying an SS-19 missile.

A similar US State Department programme plans to end by 2012, and has already stopped projects in 17 Russian and Ukrainian institutes — even though the DOE has kept its programmes in the same places. "There's reason to wonder if perhaps the IPP programme has been counterproductive," says Robinson, who supervised the report. "The Russians and Ukrainians themselves contend that it's not all that relevant to modern realities." At the hearing, Congressional representatives lashed out at the programme. "There is a thin line between the noble and the naive," said John Dingell, a Democrat from Michigan and chair of the House Committee on Energy and Commerce.

Since its inception, IPP has spent US$309 million on almost 17,000 scientists, and was given $30 million for 2008. Bart Stupak, another Michigan Democrat on the House committee, wonders if such small amounts of money, spread across so many scientists, could really deter them from selling their secrets. "$35 a day isn't going to keep anyone from doing anything," he says. The report notes Russia's recent budget surpluses and that some of its research institutes have been renovated — one has a marble foyer and a fine art collection.

Henry Sokolski, director of the Washington DC-based Nonproliferation Policy Education Center, questions the IPP's premise, saying that the US should have encouraged weapons scientists to emigrate to the West rather than "bribing" them not to sell their weapons secrets. He says the programmes have perpetuated Russian weapons complexes instead of shrinking them. But Elena Sokova, of the James Martin Center for Nonproliferation Studies in Monterey, California, supports programmes that target brain drain, and says that paying scientists to work on civilian non-proliferation research does dilute their work on weapons. The problem, she says, is real. A 2003 survey of more than 600 Russian scientists found that 20% said they would consider working for terrorists or for states that sponsor terrorism.

Adam Scheinman, the DOE's assistant deputy administrator in the office of non-proliferation and international security, says that he is working towards establishing stricter graduation criteria and is already shifting emphasis away from Russian programmes and towards scientists in Iraq and Libya. He acknowledged at the hearing that it is difficult to provide hard evidence that the programme money is keeping nuclear secrets safe, but said there is soft evidence that the programme is instilling a more ethical and transparent culture within the weapons complexes. "We are making a difference, even if I can't count it on my fingers and toes," he told legislators. ■

Eric Hand

VIRGIN GALACTIC

SPACESHIP TWO UNVEILED
Clever spacecraft design allows for training and 'safer' return to Earth.
www.nature.com/news

SNAPSHOT
Search for Higgs primed to start

CERN

The final element of the Compact Muon Solenoid (CMS) detector is gently lowered into place at CERN, Europe's particle-physics laboratory near Geneva in Switzerland. The detector will look for physics beyond the 'standard model' in the high-energy collisions of protons at the Large Hadron Collider, CERN's next-generation particle accelerator.

On 22 January, the last piece of the 1,430-tonne detector was installed in a chamber 100 metres below the surface. There, researchers will join it with 14 other segments to complete the instrument. The CMS will help to lead the search for the Higgs particle, which is believed to endow all other particles with mass. If all goes to plan, it could take its first data later this year. ■

Geoff Brumfiel

Funding freeze shakes Russia's prodigals

Hundreds of young Russian researchers are in a financial limbo after an acclaimed funding programme run by the Russian Academy of Sciences (RAS) stopped without warning a few weeks ago.

Georgii Bazykin is one researcher hit by the freezing of the programme, which allows young Russian researchers to set up independent groups in their homeland. With a PhD from Princeton University under his belt, the young geneticist returned to Moscow last summer with a generous three-year grant from the Scientific Programmes of the Presidium of the RAS. Six months later, with his funding stopped, he is in such a tight spot that he is considering returning to the United States.

The funding programme was set up in 2002 and covers 16 scientific disciplines. No explanation has been given for why the money has dried up, and it is unclear whether the programme will continue.

"We're in a very awkward situation," says Georgii Georgiev, who chairs the council of the programme in molecular and cell biology. "The programme has not been formally terminated, but the academy Presidium keeps postponing its renewal from one week to the next. People are getting very nervous here."

The RAS Presidium is presently preoccupied with redrafting the statutes of the academy and with preparing for presidential elections in May, programme officials have been told. Georgiev, who is still optimistic that the grants will continue, was told by RAS officials that a decision is due on 12 February. However, there are rumours that the RAS is considering disrupting the programme throughout most of the year, possibly terminating all ongoing projects and launching a new competition.

The programme is unique in Russia's research system in that grant winners are selected through open competition on the basis of scientific performance. The grants — up to US$180,000 per group per year — are also extremely generous by Russian standards. The programme in molecular and cell biology has been particularly successful, attracting several talented researchers back to Russia.

"I don't see how I could sustain my work and my family in Moscow without the grant," says Bazykin, whose regular salary from the Kharkevich Institute, one of more than 400 institutes run by the RAS, is just $200 per month. "I'm very concerned," he says. "If the programme were to be cut, the chances are that I will leave." ■

Quirin Schiermeier

B. KAVASHKIN/ITAR-TASS

The Russian Academy of Sciences may have cut its repatriation programme.

New York to police air monitoring

Taking stock of New York City's air and water may soon be more difficult for researchers. A new bill from the administration of Mayor Michael Bloomberg would make it a misdemeanour to possess or use a chemical, biological or radiological detector without a permit issued by the city's police department.

The legislation, which would be the first of its kind in the United States, is designed to regulate a growing number of firms that sell environmental sensors to guard against terrorist attacks. But some say that its current language is too vague and would include thousands of privately held sensors used routinely to monitor the city's air and water quality.

"At best, it would just be adding a huge level of bureaucracy and expense," says environmental geochemist Steven Chillrud of the Lamont-Doherty Earth Observatory at Columbia University in New York. Chillrud, who has studied the health effects of steel particles in the city's subways as well as air quality after the 11 September 2001 attacks, is helping to lead a move against the bill along with colleagues.

Last year, the US Department of Homeland Security approached the city's police department to discuss regulating the sensors. Police say that introducing permit requirements would ensure the sensors' quality, reduce the potential for false alarms and establish a clear alerting process in case of positive results. "For public safety reasons we need to know who is using these devices," says Jason Post, the mayor's deputy press secretary.

M. FUNK/GETTY

Change in the wind: monitoring the air on New York's Fifth Avenue may become illegal without a permit.

But the line between a detector that is specifically designed to pick up on an attack (see 'BioWatch to get second look'), and long-standing technology that may do it as a matter of course, is proving hard to define. The bill does not specify which types of detectors will require permits, nor does it exempt certain groups from permit requirements.

That flexibility, says Chillrud, means that the bill "doesn't preclude someone years from now from taking advantage of this all-encompassing language".

Of particular concern is the potential to hinder the deployment of environmental monitors in emergencies or for independent analysis of other findings. Requiring permits, Chillrud says, could also impede routine activities such as borrowing equipment from colleagues.

"It's overbroad and imposes major limitations on community organizations, academic institutions and others concerned with monitoring the environment," adds Joel Shufro, director of the New York Committee for Occupational Safety and Health, which routinely conducts indoor air-quality tests.

Although public input was not sought before the legislation was drafted, last week the police department requested comments from researchers through Columbia University administrators. "There are some problems that exist with the bill that need to be rectified," says Peter F. Vallone Jr, chair of the council's public-safety committee.

A revised draft of the bill, circulated late last week, included time-limits on the permitting process to prevent unnecessary delay. Final language for the bill may be decided on as early as mid-February, and the council plans at least one more public meeting to discuss it before it comes to a vote. ■

Rachel Courtland

BioWatch to get second look

As New York City considers permits for privately held biological sensors, the federal programme for detecting biological threats is due for a review. In spending bills approved late last year, Congress set aside $2 million for the National Academy of Sciences to review the $77-million BioWatch programme, which was set up by the Department of Homeland Security after the terrorist attacks of 11 September 2001. The review has not yet started.

So far, the five-year-old BioWatch programme has placed air-collection devices in more than 30 US cities. These devices, which hold air samples for later analysis, are poised to be replaced by a next generation of automated boxes. Twenty-six of these new boxes, which have been in development since the start of the programme, will be piloted this year in two undisclosed cities at a cost of $5.8 million.

Congress requested the review after hearing testimony last year from public-health advocates who say that the programme's budget should be invested instead in developing rapid diagnostics for hospitals, better disease reporting, and surveillance of public-health trends. But supporters of the programme say that none of these measures will mitigate the impacts of a large biological attack.

"The whole idea behind BioWatch is to detect the attack as soon as it can occur," says former Department of Homeland Security assistant secretary Penrose Albright, now the managing director of the Washington-based consulting firm Civitas Group. He says that models show that waiting for individuals to show up in the emergency room could potentially waste days and cost thousands of lives. **R.C.**

Reviewer leaked Avandia study to drug firm

A peer reviewer for *The New England Journal of Medicine* (*NEJM*) broke confidentiality and leaked a damaging report about the blockbuster diabetes drug Avandia to the drug's manufacturer weeks ahead of publication, *Nature* has learned.

Avandia (rosiglitazone) came under heavy scrutiny after 21 May 2007, when the *NEJM* published online a meta-analysis[1] of other studies into the drug's efficacy and safety. The results showed that the drug increased the risk of heart attack by 43% in people who took it for at least 24 weeks. The report garnered widespread media attention, prompted the Food and Drug Administration (FDA) to issue a safety alert, and cut the stock price of Avandia's manufacturer, GlaxoSmithKline (GSK), by 13%.

J. SULLIVAN/GETTY IMAGES

Sales of diabetes drug Avandia have fallen sharply in the wake of health concerns.

But 17 days earlier, the reviewer, diabetes researcher Steven Haffner of the University of Texas Health Science Center at San Antonio, had faxed his copy of the article to Alexander Cobitz, a GSK employee whom Haffner knew from working on an earlier clinical trial of the drug. "Why I sent it is a mystery," Haffner told *Nature*. "I don't really understand it. I wasn't feeling well. It was bad judgement." Haffner says that Cobitz did not ask to see the draft and was "probably a bystander".

> "Why I sent it is a mystery. I don't really understand it. I wasn't feeling well. It was bad judgement."

Nancy Pekarek, a spokeswoman for GSK, says that the company did not offer any input to Haffner on the meta-analysis, and that she was not aware of anyone at GSK informing the *NEJM* of the confidentiality breach.

Haffner had earlier served on the steering committee of a GSK-sponsored clinical trial of Avandia. He says that he has given many talks for the company, although he declined to say how much he had earned from them. "I've got a considerable amount of money. I didn't do it to raise my income or anything like that," he says.

After publication of the meta-analysis, GSK vigorously contested the study's methodology and "strongly disagreed" with its conclusions. Then on 5 June, again in the *NEJM* online, GSK-sponsored researchers published an interim analysis[2] of the company's RECORD trial of Avandia, a prospective study launched in 2001 to monitor adverse cardiac events. The June report referenced the May study and said that the new RECORD data "were insufficient" to determine whether Avandia increased the risk of a heart attack.

The interim RECORD analysis was published just 15 days after the damaging meta-analysis. Pekarek says that the FDA had previously asked to see the RECORD data, but that the company's inside knowledge of the meta-analysis "added an additional sense of urgency" that drove the swift publication.

Karen Pederson, a spokeswoman for the *NEJM*, says that the journal's policy is not to discuss specific peer reviewers. But she adds that any reviewer who breaks confidentiality is banned from future reviewing and from contributing editorials and review articles. Last April, the journal imposed such penalties on a prominent cardiologist, Martin Leon of the Cardiovascular Research Foundation in New York, after he talked about an embargoed study at a meeting.

In November, after being ordered to do so by the FDA, GSK added a black-box warning on Avandia packaging. The lengthy warning describes the meta-analysis and its findings, but also says that "the available data on the risk of myocardial ischemia are inconclusive".

In the third quarter of 2007, sales of Avandia were down 38% from a year earlier worldwide and down 48% in the United States. ■

Brian Vastag

1. Nissen, S. E. & Wolski, K. *N. Engl. J. Med.* **356,** 2457–2471 (2007).
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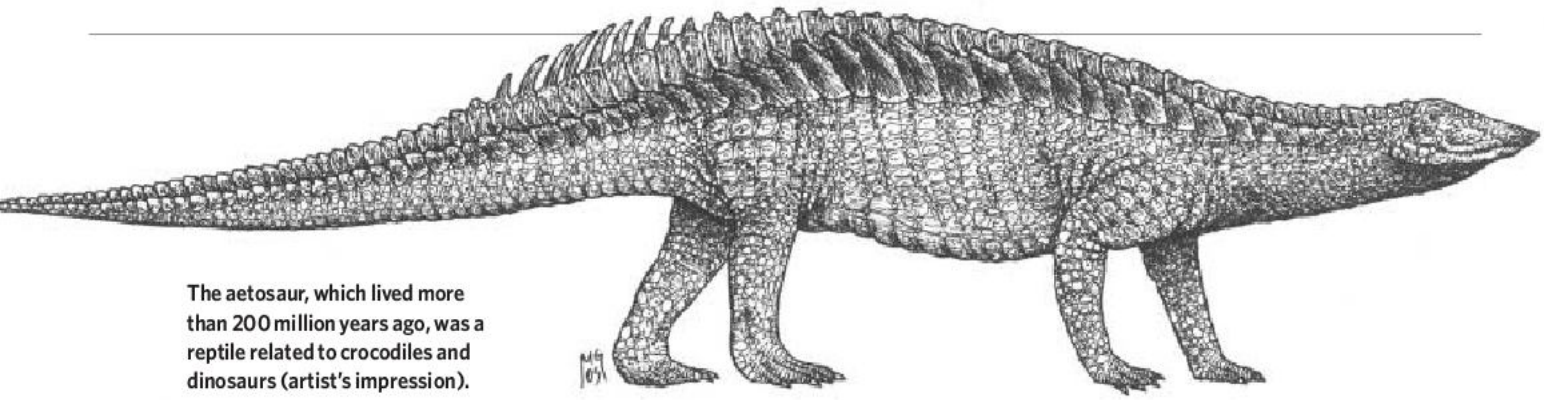

The aetosaur, which lived more than 200 million years ago, was a reptile related to crocodiles and dinosaurs (artist's impression).

Fossil reptiles mired in controversy

An ethics row has broken out among palaeontologists over the naming of aetosaurs, a type of ancient armoured reptile.

Doctoral students in the United States and Poland are accusing scientists at the Albuquerque-based New Mexico Museum of Natural History and Science (NMMNHS) of publishing articles that allegedly pilfered their research. The allegations concern three articles published in the *NMMNHS Bulletin* by the museum's interim director Spencer Lucas, former director Adrian Hunt and their co-authors.

The disputed articles name and describe different aetosaurs, and detail how the 220-million-year-old reptiles are related to crocodiles and dinosaurs. In one instance, Lucas, Hunt and Justin Spielmann, the museum's geoscience collections manager, are accused of rushing to publish a new name for an aetosaur (*Rioarribasuchus*)[1] when they allegedly knew that palaeontologist William Parker of the Petrified Forest National Park in Arizona was soon to publish an article naming the species (as *Heliocanthus*)[2].

The International Commission on Zoological Nomenclature says scientists must not name species if they know a competing scientist is in the process of doing so. Lucas denies knowing of Parker's plans. Hunt, who left the museum last July to head the Flying Heritage Collection being set up by Microsoft co-founder Paul Allen in Everett, Washington, did not comment.

And last July, Jerzy Dzik of the Palaeobiology Institute at the University of Warsaw sent Lucas an e-mail in complaint after Lucas published an article in the *Bulletin* describing Polish aetosaur fossils[3]. The article appeared shortly after Lucas had visited the Warsaw Institute, when the fossils were close to being described by scientists there. Such a thing had not occurred in the past 50 years at his institute, Dzik wrote, adding: "Your action was harmful to many young researchers."

In an e-mail response to Dzik, Lucas blamed the Polish researchers for not being more explicit about their fossil-examination rules, but he did apologize for what he called "a misunderstanding".

Another article published in the *Bulletin* by Spielmann and his bosses involves a reinterpretation of an aetosaur called *Redondasuchus*[4]. Jeff Martz, a palaeontology doctoral student at Texas Tech University in Lubbock, says this reinterpretation — involving bony spikes along the animal's back — failed to properly credit his own similar description in a master's thesis, an act akin to plagiarism.

In a letter of complaint sent in 2007 to New Mexico government officials, Martz, Mathew Wedel of the University of California at Merced and Michael Taylor of the University of Portsmouth, UK, wrote: "It is our strong suspicion the [New Mexico Museum team members] deliberately abused their editorial powers to take credit for observations and insights of Parker and Martz." Such actions, the letter argues, corrupt the scientific process and harm young researchers. Because Lucas largely edits the *Bulletin*, he and his team have been able "to mass produce essentially self-published and non-peer-reviewed papers", the letter claims.

B. MUELLER/MUS. TEXAS TECH

Interlocking bony plates covered the aetosaur's body, shown one-third of actual size.

Lucas is known in the palaeontology community for his desire to publish a high volume of papers. He acknowledges that his "tough" approach has brought him into conflict with researchers before. "They are obviously angry," he says, but the complaint "doesn't have any substance".

The New Mexico cultural-affairs department, which oversees the museum, conducted a review of two of the instances last October and concluded that the allegations were groundless. But some experts call that review a whitewash, claiming that it failed to follow accepted practices of US academic institutions faced with claims of misconduct. Now all three cases are before the Ethics Education Committee of the Society of Vertebrate Paleontology, a professional organization based in Northbrook, Illinois, which is awaiting responses from the New Mexico team before making a ruling.

"What we sought is a point-by-point response to our allegations," says Martz. Attorneys for New Mexico state blocked such a response, according to Peter Gerity, vice-chairman of the museum's advisory board who is also vice-president for academic affairs at New Mexico Tech in Socorro. Gerity says he helped review the 2007 letter of complaint, which was rejected. Gerity told *Nature* he was unaware of the Polish criticism.

With Lucas now seeking to become permanent director of the New Mexico museum, the publishing debate isn't expected to go away. ■

Rex Dalton

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Kidney expert to head holistic-medicine centre

A specialist in kidney disease with a keen interest in non-traditional therapies has been named as the new head of the National Center for Complementary and Alternative Medicine in Bethesda, Maryland.

Josephine Briggs says she grew interested in alternative therapies while overseeing grants at the National Institute of Diabetes and Digestive and Kidney Diseases, where she launched studies on whether cranberry juice helps prevent urinary-tract infections and on the effects of saw palmetto in men with enlarged prostate glands.

Briggs says she isn't worried about disrespect from more traditional researchers. "Some of my friends roll their eyes, but I can deal with that," she says. "It's very important that this work be done and be done well."

Early-warning system underestimates quake

In October, Japan launched its nationwide system to warn people about strong earthquakes before they hit. But the system underestimated the strength of last week's quake near the nation's west coast.

The system is supposed to issue a warning, seconds before the shaking arrives, through television or loudspeakers to people in areas where the shaking will be a 'lower 5' or more on the Japanese system of seismic intensity. The scale, which ranges up to 7, measures the level of shaking in any individual region, rather than at the epicentre of the quake. A lower 5 can break windows, put cracks in older buildings and knock over bookshelves.

KAZUHIRO NOGI/AFP/GETTY IMAGES

Are there cracks in Japan's quake warning system?

For last week's quake, the early-warning system predicted a shaking of intensity level 4, but some coastal cities experienced shaking in the range of a lower 5. Takahito Nishimiya, a senior scientific officer in the seismology division at the Japan Meteorological Agency, says that such variation is within expected limits.

Synthetic genome paves the way to artificial life

The genome of the pathogenic bacterium *Mycoplasma genitalium* has been stitched together from scratch, creating a full set of instructions to make a living thing in the lab.

Hamilton Smith and his colleagues at the J. Craig Venter Institute in Rockville, Maryland, put together the 582,970 nucleotide bases — the building blocks of DNA — that constitute the *M. genitalium* genome. The genome is more than a factor of ten longer than the previous longest stretch of genetic material created by chemical means.

Next the team aims to find out whether cells can be 'booted up' into action when loaded with the genetic program.

France and India to expand scientific collaborations

Nuclear scientists in India and France will be working more closely together after several agreements were signed on 25 January during French President Nicolas Sarkozy's visit to New Delhi.

Indian researchers will participate in the construction of the Jules Horowitz research reactor, being built in Cadarache, France. They will also work with scientists at the GANIL heavy-ion accelerator in Caen.

Under another agreement, a neuroscience laboratory will be set up in India by India's National Brain Research Centre, the University of Paris and French medical research centre INSERM.

Delhi University and Grenoble University will set up two new joint master's programmes, in nanosciences and nuclear engineering. And additionally, India's Council of Scientific and Industrial Research and its French counterpart the CNRS will partner in green-chemistry approaches to find therapeutic agents for cancer and neurological disorders.

OncoMed scores drug deal for $1.4 billion with Glaxo

The UK pharmaceutical giant GlaxoSmithKline has secured a US$1.4-billion, five-year deal with the biotechnology company OncoMed Pharmaceuticals in Redwood City, California, to develop therapies based on cancer stem cells.

The deal, among the most lucrative ever for any preclinical company, has a certain risk because the cancer stem-cell theory is not universally accepted. The theory holds that tumours develop from specific stem cells that fail to be eliminated by chemotherapy and radiotherapy and thus reseed the cancer even after a long remission.

OncoMed has developed monoclonal antibodies to target and destroy such cancer stem cells. GlaxoSmithKline has secured the rights to four of them, which will now enter clinical trials.

Correction

In the News story 'Huge crystal baffles chemists' (*Nature* **451**, 383; 2008), we mistakenly referred to the 'baubles' as the largest single-molecule crystals; in fact they are the largest crystallized single-molecule metal clusters.

Gates foundation gives cash for agriculture in Africa

AGRA

Agriculture in developing countries got a big boost last week, when the Seattle-based Bill & Melinda Gates Foundation announced it would spend US$306 million on projects aimed at improving agricultural productivity.

The bulk of the money — $164.5 million — will go to the Alliance for a Green Revolution in Africa, in Nairobi, Kenya, which will use it to fund projects to rejuvenate nutrient-depleted soils over 6.3 million hectares of African farmland.

The rest will be split between outfits that develop micro-irrigation technologies for smallholders in India, boost high-quality coffee production and milk quality in Kenya and elsewhere, and improve dairy farmers' access to markets in Bangladesh. The International Rice Research Institute in Manila, the Philippines, will receive $19.8 million over three years, the largest single injection of money into rice research for several decades.

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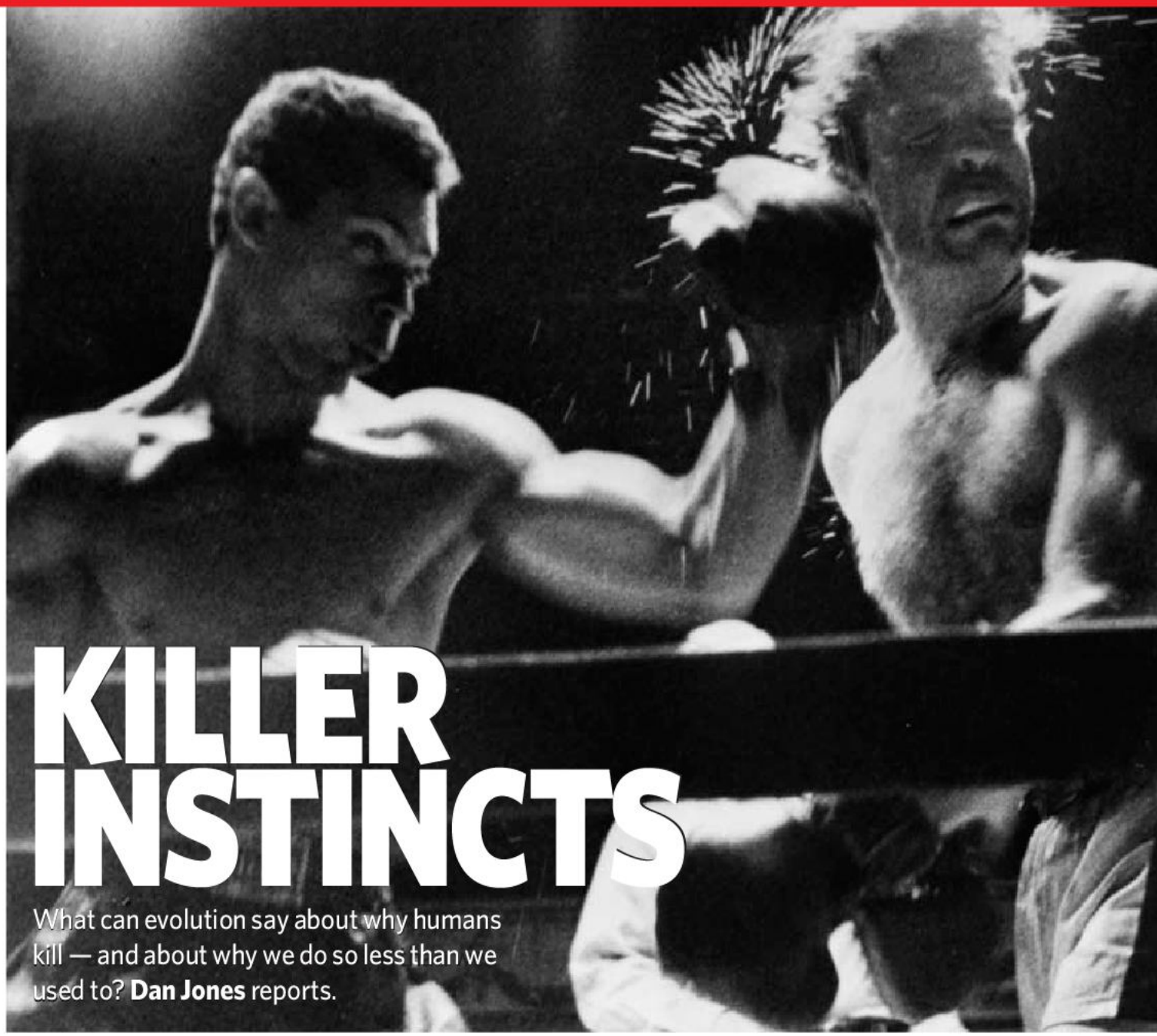


KILLER INSTINCTS

What can evolution say about why humans kill — and about why we do so less than we used to? **Dan Jones** reports.

MGM/UA/PHOTOFEST

"It is scientifically incorrect to say that we have inherited a tendency to make war from our animal ancestors … that war or any other violent behaviour is genetically programmed into our human nature … [and] that humans have a 'violent brain.'"

These are the ringing words of the 'Seville Statement on Violence', fashioned by 20 leading natural and social scientists in 1986 as part of the United Nations International Year of Peace, and later adopted by the United Nations Educational, Scientific and Cultural Organization (UNESCO). It was written to counter the pessimistic view that violence and war are inevitable features of human life.

The decades since have not been kind to these cherished beliefs. A growing number of psychologists, neuroscientists and anthropologists have accumulated evidence that understanding many aspects of antisocial behaviour, including violence and murder, requires the study of brains, genes and evolution, as well as the societies those factors have wrought.

At the same time, though, historians, archaeologists and criminologists have started to argue that in most places life was more violent — and more likely to end in murder — in the past than it is today. The time span of this apparent decline in violence has been too short for appeals to natural selection to be convincing. If humans have evolved to kill, then it seems that they have also evolved to live without killing, given the right circumstances.

Going too far

Just two years after the Seville Statement was issued, Martin Daly and Margo Wilson of McMaster University in Ontario, Canada, published *Homicide*[1]. The book was to become one of the founding texts of a new — or at least thoroughly rebranded — discipline called evolutionary psychology. Drawing on animal behaviour, anthropology and patterns of violence and murder in modern societies, Daly and Wilson provided an evolutionary account of the various forms of homicide, from one man killing another to spousal murder and the rarer killing of step-children. But although they argued — in direct contradiction of the Seville Statement — that humans have brains and minds with violent proclivities, they also argued that killing was, by and large, not something that evolution had selected for.

Instead, Daly and Wilson argued that murderous actions are usually the by-product of urges towards some other goal. The purpose of the sometimes violent competition that goes with human urges for higher status and greater reproductive success is not to kill, any more than the purpose of its stylized quintessence boxing is. But sometimes people die.

Most evolutionary psychologists agree, in general terms, with this 'by-product' view, although there are exceptions. David Buss, of the University of Texas at Austin, and Joshua Duntley, of the Richard Stockton College of New Jersey in Pomona, have developed a controversial 'homicide adaptation theory'. The theory proposes that, over evolutionary history, humans have repeatedly encountered a wide range of situations in which the benefits of killing another person outweighed the

costs — particularly when the assessed costs of murder are low, success is likely and other non-lethal options have been closed off[2]. The killing of an unwanted child or the stealthy murder of a sexual rival might be examples. "Homicide can be such a beneficial solution to adaptive problems in certain, specific contexts that it would be surprising if selection had not fashioned mechanisms to produce lethal aggression," says Duntley. Other evolutionary psychologists are yet to be convinced. "I wouldn't want to hitch my wagon to the by-product argument," says Daly, "but I don't think anyone, including Duntley and Buss, has figured out a good way to identify the hallmarks of homicidal adaptations."

A key condition for an evolutionary account of homicide is an explanation of the fact that most deadly violence is committed by men. Evolutionary psychologists say that this is because men have evolved to compete more intensively than women in the race for status, material wealth and sexual partners. In terms of the by-product theory, men are more likely to suffer the consequences when competition gets out of hand. This competitive kindling, Daly and Wilson argue, is at its most combustible in men of low socioeconomic status in regions of high social inequality, suffused with a sense of everything to gain and little to lose.

Although women also compete, they may be less likely to do so in ways that risk escalating to the use of deadly force because, for women, the costs of such escalation have historically been higher. Rebecca Sear at the London School of Economics and Political Science and Ruth Mace of University College London recently studied the effects of losing kin on child survival in 28 populations from around the world over the past three centuries[3]. The death of a mother has an impact on child survival — but often the death of a father does not. From a gene's eye view, a woman who might die is thus a bigger problem than a man facing the same level of risk.

A meta-analysis[4] of studies looking at sex differences in aggression by John Archer of the University of Central Lancaster, UK, reveals that men and women don't differ much in their experience of anger, the primary accelerator of aggression. Anne Campbell, an evolutionary psychologist at Durham University, UK, suggests that the differences in aggressive behaviour thus reflect differences in the strength of the factors controlling the behavioural expression of that anger. "Developmental studies show that girls generally score higher on empathy measures, are more fearful and are better at controlling their behaviour," says Campbell.

In crude terms, women may in general have better brakes with which to stop a violent impulse and people who are violent may, in general, lack such brakes. Psychologist and neuroscientist Richard Davidson, of the University of Wisconsin-Madison, suggests that dysfunction in the brain circuits that normally inhibit emotional impulses — those associated with the prefrontal cortex — is a crucial prelude to violent outbursts[5].

> "I don't think anyone has figured out a good way to identify the hallmarks of homicidal adaptations."
> — Martin Daly

In 1997, Adrian Raine and Lori LaCasse, then at the University of Southern California (USC) in Los Angeles, and their colleague Monte Buchsbaum from Mount Sinai School of Medicine in New York published one of the first explanations of the neurobiology of homicide. Among the brains of 41 murderers pleading not guilty by reason of insanity, they found lower activity (as measured by glucose metabolism) in the prefrontal cortex, and greater activity in structures in the limbic system, thought to drive aggression, than they found in non-murderous brains[6]. "Put crudely, murderers don't have the prefrontal resources to regulate that unbridled emotional output," says Raine.

Raine has since found a link between a lower volume of grey matter in an area of the prefrontal cortex known as the orbitofrontal cortex, which has been associated with decision-making and regulation of emotion, and more aggressive and antisocial behaviour. He says that the difference in the average volume of the orbitofrontal cortex between men and women accounts for about half of the variation in antisocial behaviour between the sexes. Just as evolution has shaped men's bodies to be, on average, larger than women's, it has also distributed the resources needed to regulate emotion and aggression unevenly between the sexes.

In an intriguing turn, Raine and his USC colleague Yaling Yang have recently pointed to a link between homicidal behaviour and the capacity to follow moral guidelines. Over the past six years, brain-imaging studies aimed at understanding moral judgements have illustrated the crucial role of the emotional feeling that comes with violating moral codes. Parts of the prefrontal cortex and amygdala that are abnormal in violent individuals and murderers are activated when making moral judgements. Raine and Yang have proposed that these systems serve as the engine that translates moral feelings into behavioural inhibition — an engine that has blown a gasket in the antisocial, violent and murderous[7].

A lethal legacy

Men are not just more likely to kill other people than women are, they are also more likely to do so in groups — and for some researchers it is in these realms that killing offers real evolutionary value. The murder of one person by another may be almost accidental, an unlooked for by-product of aggression. The murder of members of one group by those of another could be an adaptive behaviour that evolution has encouraged.

Humans are not the only primates to form coalitions that kill members of neighbouring communities. Since the behaviour was first reported at the Jane Goodall research centre in Gombe, Tanzania, in the 1970s, five long-term study sites dotted around Africa have seen murderous 'gang violence' in chimpanzees. In one case that is hard not to see as a war, the adult males of one community systematically attacked and killed the males of another group over a period of years, with the victorious group eventually absorbing the remaining victims.

Harvard anthropologist Richard Wrangham

has been observing primates in the wild for more than 30 years. He thinks that the roots of chimpanzee warfare lie in the social organization and behavioural ecology of their societies. Although chimps live in communities of around 150, they are rarely all found together. Instead they typically travel around their territory in parties of up to 20 animals. From time to time, a roaming party from one group will cross paths with a roaming party of another. If they are of equal size, there will be a lot of screaming and charging. When there is an imbalance of power, the larger party will often try to isolate and attack an enemy chimpanzee, sometimes holding their victim down while the frantically excited attackers hail down lethal bows.

Although these attacks can be risky — small parties have been seen running to attack a lone neighbour only to find themselves surrounded by a much larger party, at which point they hurriedly try to flee — they can also have big pay-offs, especially over the long-term. By dominating or eliminating neighbouring communities, aggressors can expand their range, which means a better food supply, healthier adults and faster reproduction[8].

Raids on neighbouring communities are also common in anthropologists' accounts of small-scale human societies. These often follow the chimpanzee template: a small band of men leaves its home ground, sneaks up on the neighbours and tries to kill one or more of them. Wrangham, working with Michael Wilson of the University of Minnesota in St Paul and Martin Muller of the University of New Mexico in Albuquerque, has moved beyond remarking on the general similarity to looking at some real numbers. They compared death rates from conflict between groups of chimps in the five long-term study sites[9] with data for inter-group human conflicts in numerous subsistence-farmer and hunter–gatherer societies assembled by anthropologist Lawrence Keeley of the University of Illinois at Chicago. Overall, humans and chimpanzees showed comparable levels of violent death from aggression between groups.

> "In major international wars people do what they do mainly because it is their duty in the role they occupy."
> — Robert Hinde

A history of violence

Moving from studies of chimpanzee coalitional violence and comparisons with small-scale tribal conflicts to understanding modern warfare is, however, far from straightforward. 'War' is a broad term, points out Robert Hinde, a zoologist at the University of Cambridge, UK, and one of the signatories to the Seville Statement. Although Hinde mostly agrees with Wrangham's take on the parallels between inter-community conflicts in chimpanzees and humans, he has reservations about extrapolating too much from these studies. "In major international wars people do what they do mainly because it is their duty in the role they occupy; combatants in institutionalized wars do not fight primarily because they are aggressive," says Hinde, who served as a fighter pilot in the Second World War.

But some of the normal machinery that inhibits violence — the moral engine described by Raine and Yang — might become selectively disengaged in warring armies. Ideology, propaganda and denigration of the out-group can harden the barrier between 'us' and 'them', says Hinde — a barrier to which the mind's moral faculty is very sensitive. As a result, killing comes to feel permissible. Even, sometimes, right.

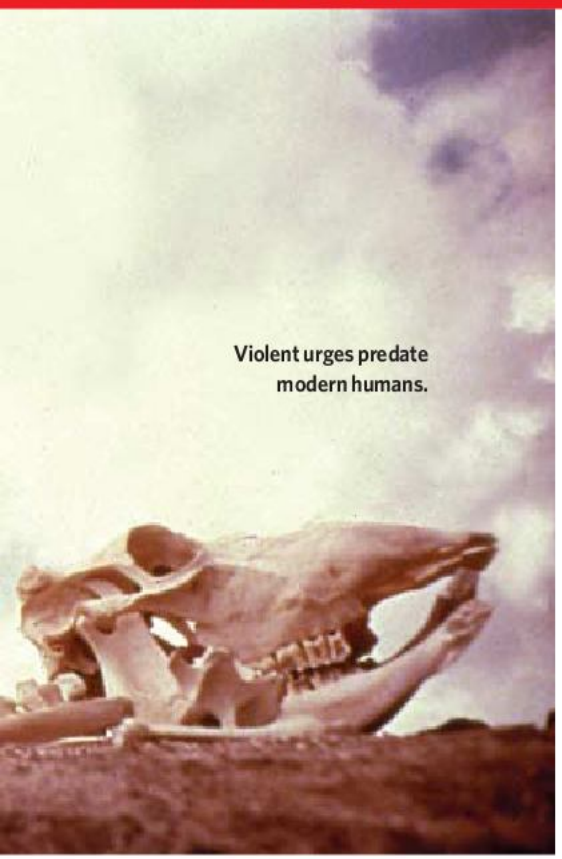
What about comparisons of aggression and killing within groups? Chimps often turn on their own, particularly infants and young adults. According to Wrangham and his colleagues, in-group killing exceeds death from between-group conflict in at least some chimp communities. Humans in small societies, by contrast, die much less frequently from fights within their group than from group battles. One possible explanation is that they simply fight less. Anthropologist Victoria Burbank of the University of Western Australia in Crawley has recorded[10] rates of non-lethal acts of physical aggression in an aboriginal Australian population; by Wrangham's reckoning, chimps display such behaviour 200 times more frequently, if not more.

For an increasing number of behavioural scientists, including Hinde, this prosocial lack of violence looks like a fundamental aspect of human nature — the human ability to generate in-group amity often goes hand in hand with out-group enmity. Using computer simulations, economists Jung-Kyoo Choi from Kyungpook National University in South Korea and Samuel Bowles from the University of Siena in Italy have produced models in which altruism and war co-evolve, promoting conflict between groups and greater harmony within them[11]. "It all falls into place when you see the evidence that early humans lived in small, competing groups," says Hinde. "Your group was more successful if you cooperated with its members but not with outsiders."

None of this means that a tendency to kill is set in stone; if anything, it shows that humans have evolved to be much less of a risk to each other within groups than they would be if they were as bellicose as chimps. And there is evidence that this risk is reducing further in studies of death rates from both inter-group homicide and intra-group warfare, both of which seem to have plummeted over the millennia.

Incessant tit-for-tat tribal raids in which a high percentage of people took part led to shocking rates of death at human hands, spears, axes and clubs. Harvard psychologist Steven Pinker was relying on estimates of this violence derived by anthropologists when he suggested that "if the wars of the twentieth century had killed the same proportion of the population that die in the wars of a typical tribal society, there would have been two billion deaths, not 100 million."[12]

A decline in inter-personal violence (as opposed to inter-group war) can be seen over the shorter timescale and narrower field of modern European history. Criminologist Manuel Eisner at the University of Cambridge



Violent urges predate modern humans.

MOVIESTORE COLLECTION

has documented a trend of declining homicide rates estimated from historical records left by coroners, royal courts and other official sources spanning Europe from the twelfth century to the modern day[13]. After rising from an average of 32 homicides per 100,000 people per year in the thirteenth and fourteenth centuries to 41 in the fifteenth, the murder rate has steadily dropped in every subsequent century, to 19, 11, 3.2, 2.6 and finally 1.4 in the twentieth century. England is typical of the trend, going from 23 homicides per 100,000 people per year to 1.2 over the same period.

Eisner rules out better policing and improved medical treatment as causes of the decline for the simple reason that it started before professional police forces appeared and techniques for dealing with wounds became more effective. And a few centuries is too short a time for evolution to have shaped human nature much. A part of the answer that is consistent with an evolutionary approach is a long-term reduction in inequalities of life circumstances and prospects — the inequalities that Daly and Watson see as driving the conflict that leads to killing as a by-product. "In places such as Sweden where every cabbie drives a Mercedes," says Daly, "people don't bother to kill so often." Better provisioning of life's necessities has also powered the decline, agrees Duntley. When contested resources are made more plentiful, he says, conflict over resources decreases and homicide rates drop.

Moral rearmament

The picture, though, is hardly simple. Societal specifics play a part as great as or greater than that of any evolutionary generalities. Eisner points out that across Europe, both geographically and through time, countries with the highest homicide rates are typically plagued by familial feuding and blood revenge, such as in the Scottish highlands in the eighteenth century and Sardinia in the nineteenth. The death toll was frequently exacerbated by cultures laying weight on a male strength in arms and a willingness to demonstrate it. Perhaps against the spirit of Daly's argument, violence was particularly prevalent in élites, who would often use it with impunity against their social inferiors. "Violence is a very functional thing, and the élites used it to their advantage," claims Eisner, pointing out that violence as a phenomenon of lower-class youths — the sort of violence Daly and Wilson have studied in Chicago crime statistics — is a recent trend.

"In the early modern period, local élites and nobility become integrated into the state and they increasingly find violent and aggressive behaviour to be useless or dysfunctional," says Eisner. "It becomes much better to be economically successful, and so the élites abandon their violent behaviour." Systems of justice in which the right people are pardoned and the right people punished push up the costs of violence and homicide, and can put an end to the otherwise incessant family feuds. They can also provide clean alternatives to the shedding of civil blood. According to Eisner, European records reveal that 10–20% of medieval homicides were related to conflicts over land ownership. "Administrations that determine who owns what, and access to civil law courts that help you resolve disputed claims, make resorts to violence much less likely — in a modern society, it's actually counterproductive," he says.

A drop-off in war could also lead to reductions in other forms of violence. In cultures and societies with a recent history of warfare, children tend to be socialized to tolerate pain and to react aggressively, which prepares them for the possibility of becoming a soldier (arguably something that evolution would favour) or a potentially deadly brawler (probably something it wouldn't). But in much of the world, histories of warfare are becoming more distant. "If we grow up without these experiences, which is the case for most people in modern democracies that could affect how aggressive we are and our moral views of our options," says Wilson.

The evidence suggests that humans may indeed have what the Seville Statement called a 'violent brain', in as much as evolution may favour those who go to war. But evolution has also furnished us with a moral sense. The complexities of the relationship between morals and violence may prove a fruitful field for future research, in as much as they can be disentangled from the social and historical factors that clearly hold great sway over the ultimate levels of violence. Evolution is not destiny; but understanding it could help maintain the hard-to-discern progress of peace. ■

Dan Jones is a freelance writer in Brighton, UK.

Killing and camaraderie could have co-evolved.

WARNER BROS/THE KOBAL COLLECTION

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Genetics by numbers

Genomewide association studies are starting to turn up increasingly reliable disease markers. **Monya Baker** investigates where we are now and what comes next.

W. FERNANDES

Who would have thought that the future of human health would read like a list of car number plates? Last year, a suite of studies[1–3] pinned an increased likelihood of developing heart disease on some mysterious culprits: seemingly incomprehensible strings of numbers such as rs10757274 and rs1333040. The number sequences, technically known as single nucleotide polymorphisms or SNPs, are located close to one another on chromosome 9. No one knows what they do, if anything. But carrying two copies of any of them boosts a person's chance of developing heart disease by the same amount as smoking ten cigarettes a day. The effect is less than that brought on by diabetes or heavier smoking, but it is still one of the strongest risks so far identified by genomewide association studies, which attempt to find genetic variants that occur more frequently in one group of people than another.

The association is so robust that it is already being used as a positive control for further genomewide studies of cardiovascular disease. "If you don't find it, you know something is wrong" with the analysis, says Ruth McPherson of the University of Ottawa Heart Institute in Canada and lead author on one of the two articles that first announced the association[1] in May 2007. Researchers from deCODE Genetics in Reykjavik, Iceland, published the other[2]. Having one copy of any of the wrong SNPs boosts risk by about 40%. Having two copies of the SNP doubles one's chances of having a heart attack early in life.

> "The associations are so robust, that if you don't find them, you know something is wrong."
> — Ruth McPherson

But despite the strength of the association, pegging meaning to it is difficult. Genomewide association studies find SNPs, single letter changes in the genome, that appear commonly and may correlate to more variation in nearby DNA. SNPs are not necessarily the causative mutations. They might not even be in gene-coding or gene-controlling regions, and thus may not contribute, even indirectly, to risk. The heart-risk SNPs indicate only that the culprit is located within a long genomic region on chromosome 9 known as 9p21.3. No protein-coding genes are apparent within the region, and investigations of two nearby genes — both tumour-suppressors — haven't yielded an explanation.

Making sense of the numbers

Still, enthusiasts of genomewide association can't wait to find more mysteries like rs10757274 and rs1333040. Scanning hundreds of thousands of SNPs for a disease or condition can turn up dozens of associations. Early studies gained notoriety for their unreproducible results, turning up many false positives. But that is changing with increasing statistical savvy and larger populations for whom more SNPs have been measured. Last year, replicated studies identified loci associated with common diseases such as type 2 diabetes, Crohn's disease and cardiovascular disease[4] (see 'SNP spotting') leading some to call 2007 the year of genomewide association studies, as research groups pumped out dense lists of associated SNPs and gene regions like so many car number plates — meaningless without further information. Ask researchers what is next for 2008 and 2009, and they will gush about longer lists. As researchers scan larger populations for more SNPs, more SNPs will be associated more reproducibly with more diseases.

But ask when these obfuscated strings of digits and letters will be used to help find a drug, explain a disease, or recommend tailored treatments for patients, and you'll get a sober response that years of work lie ahead. Researchers readily admit that SNP-scanning studies cannot find important contributors to disease, such as environmental factors or extra copies of genes. Moreover, identified variants, or alleles, contribute to only a small part of the overall risk. Still, by scanning the entire genome, association studies can uncover unsuspected connections between genes and disease. "Finding the initial SNP is not the same as finding the underlying biology," says human geneticist David Altshuler of the Broad Institute in Cambridge, Massachusetts, "but it's a big step forward."

Companies are already selling or planning to sell tests that scan for genetic variants associated with disease. Last November, deCODE began selling a test to doctors that will reveal how many copies of the risk allele at 9p21.3 an individual carries. Those kinds of tests worry Muin Khoury, director of the National Office of Public Health Genomics at the Centers for Disease Control and Prevention in Atlanta, Georgia, who has written cautionary commentaries on the issue. "Even if the association is replicated in many, many studies," he

says, "it is still weak information." Right now, family history is more predictive. There is no evidence that results of genetic tests encourage people to adopt healthier lifestyles, Khoury says, and such tests could even yield false hopes, as someone who lacks identified risk alleles could be carrying risk variants that are as yet unidentified. Evidence that they make a difference may be a long time coming. "The private sector doesn't want to do the research and right now no one is telling them they have to," Khoury says.

Cooperation is key

Nevertheless research continues to seek out new associations in the hope of developing a richer understanding of disease. Ultimately, common variants of even very important genes will have small effects; finding them depends on having enough data to sort through. Genomewide association studies are expensive. Although prices have plummeted, genotyping a few hundred thousand SNPs still costs a few hundred dollars per individual, not counting the complex and expensive tasks of collecting and managing patient data. "Sample size has been our big enemy here, and sharing data is the best way possible to address this issue," says Lon Cardon, a statistical geneticist at the University of Washington in Seattle.

To boost sample sizes, the Wellcome Trust has created its Case Control Consortium (WTCCC) and assembled 2,000 patient samples for each of several diseases along with a generic set of 3,000 controls, some 19,000 subjects in all. The rationale is that by comparing SNPs between diagnosed and undiagnosed individuals, genetic risk associations will be apparent.

Missing the point

Nilesh Samani, chair of cardiology at the University of Leicester, UK, and one of two lead investigators responsible for coronary heart disease with the WTCCC, explains that even studies with many samples will miss variants with modest effects. Suppose, he says, that there are 10 loci in a genome that each increase the likelihood of a condition by 20%. Statistically, an examination of 2,000 cases and 2,000 controls would pick up at most three of these loci. An independent group with similar sample sizes might also find two or three loci, but they might be different loci, and the plague of false positives would make results inconclusive. "It's only when we pool all of these studies together that we have a realistic chance of picking up all of those loci," Samani says.

The data provided by the WTCCC are largely limited to genotype, age, gender and presence of disease. They are proving invaluable for certain studies, but finding how variants affect specific traits such as cholesterol levels or blood pressure requires richer data. A particularly rich vein of it came from the Framingham SNP Health Association Resource (SHARe), which genotyped 550,000 SNPs in more than 9,000 individuals participating in the Framingham Heart Study.

Sponsored by the National Heart, Lung and Blood Institute in Bethesda, Maryland, the 60-year-old Framingham study has followed three generations of individuals in Massachusetts. Data from it established smoking and high cholesterol as risks for heart disease. Although not all phenotypic data exist for every individual, the data set contains thousands of clinical variables from blood analyses to vascular imagery to lifestyle surveys, sometimes from the same individual over a span of years. Assembling the database required Framingham to undertake a sort of archaeology expedition of old medical records and publications, but, as the acronym implies, the data are available to other researchers.

> "Finding the initial SNP is not the same as finding the underlying biology."
> — David Altshuler

As of 25 January 2008, all genomewide association studies funded through the US National Institutes of Health (NIH) are required to deposit their data in the Database of Genotype and Phenotype (dbGaP). Although researchers who collect the data have exclusive rights to publish their analyses for at least nine months, scientists who promise to uphold privacy safeguards will be able to access others' data sets immediately. SHARe has a similar moratorium.

Such practices have become standard in genomics studies. Nevertheless, some worry that if scientists can publish analyses of downloaded data, they might have fewer incentives to collect the data necessary for answering new, interesting questions. They may also not properly

SNP spotting

DIABETES
Initially identified and confirmed using samples from more than 32,500 individuals, the association between rs10811661 and diabetes was first reported[9] in 2007. It is found on chromosome 9 near a gene that — a study in mice showed — can prevent pancreatic islet cells from regenerating when the gene is overexpressed[10]. A subsequent study found that people with the risk SNP released less insulin than is normal when they were given glucose[11].

CROHN'S DISEASE
On the long arm of chromosome 2, rs2241880 has popped up in several genomewide association studies[12-13] for Crohn's disease, but not for other gut inflammatory disorders such as ulceritis. The SNP lies within the coding region of a gene that is expressed in the lining of the intestine and helps to process intracellular bacteria. In cell culture, cells that did not express this gene were less able to defend against infecting *Salmonella* bacteria.

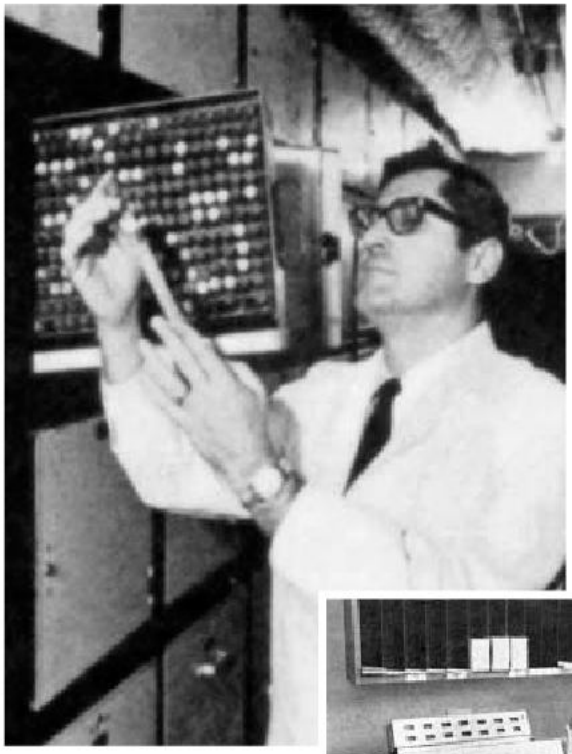
CANCER
Multiple studies[14-15] have found SNPs along a gene-poor stretch of the long arm of chromosome 8 known as 8q24. These have been associated with breast, colorectal and prostate cancer. One, rs16901979, was first found in Icelandic populations and then in African-American men. No one knows the mechanisms behind the conditions, but the region wouldn't have been found without blindly looking for associations.

handle the data they do get. Bruce Psaty, a cardiologist and epidemiologist at the University of Washington in Seattle, examined how researchers used data available from two large studies. He found that not only did some researchers go beyond the scope of original agreements, they failed to account for the study's design in their analyses. For example, some analyses searching for predictors of a patient's first heart attack did not exclude patients who had already had heart attacks[5]. Poor analyses of large epidemiological studies could mean real associations are missed or false ones found. Resources are wasted either way.

Share and share alike

If scientists are careful and peer reviewers rigorous, giving more researchers access to more data should mean better science at lower prices, says Altshuler. "No single group can make full use of such large data sets. The creativity of the whole world has to be greater than the creativity of the group that collected the data." Kári Stefánsson, chief executive of deCODE Genetics, says that researchers are already doing a good job of finding collaborators but he resents what he calls the "Soviet flavour" of the NIH mandate. "I don't want to share my data with anyone because the NIH decides I should," he says. "I want to do it because I decide to do it."

Teri Manolio, director of the Office of Population Genomics of the National Human Genome Research Institute in Bethesda, Maryland, acknowledges such concerns but says that science will adjust. The WTCCC and the Genetic Association Information Network (a public–private US collaboration with goals similar to those of the WTCCC) both grew out of the Human Genome Project. As that 'big-science' project got under way, she recalls, there were "big concerns" that people's careers would end when they put their data on the web. That didn't happen. "It quickly became apparent that just putting a sequence up wasn't a publication, and that that kind of data-sharing didn't actually hurt people," says Manolio. In fact, she adds, some initial contributors to the dbGaP have gained additional collaborators and recognition by sharing data, and several academic and corporate investigators plan to contribute data even though they are not required to.



FRAMINGHAM HEART STUDY

FRAMINGHAM HEART STUDY

William Kannel (above) was the second director of the labour-intensive (inset) Framingham Heart Study.

With the need for large data sets and the danger of false-positives now both widely recognized, Cardon hopes that scientists can be relied on to do what is necessary to conduct interesting, convincing analyses. "I think it is really useful to put data up on dbGaP, but it is no substitution for collaborating with the people who collected them," he says. "It's certainly more powerful than just downloading and trying to go it alone."

Strength in numbers

When multiple data streams come together, results are tangible. After surveying hundreds of thousands of SNPs from 2,758 individuals for associations with blood lipid levels, researchers working with data from the Diabetes Genetics Initiative had settled on 196 SNPs for further analysis. Then they were contacted by another group with data from two more studies that had measured SNPs and blood lipid levels in 6,058 people.

> "I don't want to share my data with anyone because the NIH decides I should. I want to do it because I decide to do it."
> — Kári Stefánsson

Sharing data helped both groups identify more SNPs for further analysis, resulting in publications from both groups[6,7]. One SNP identified in that collaboration[7], rs599839, on the short arm of chromosome 1, was found to be associated with lower levels of low-density lipoprotein cholesterol, and further study showed increased expression of three genes in the liver, including one involved in glucose uptake. Interestingly, that SNP had also been associated with heart disease just months earlier, in a study[3] that confirmed the risk on 9p21.3.

Work to understand SNPs' influence will mean chasing down more informative phenotypes and, eventually, lab work to confirm function. Recently, deCODE found that the SNPs on the 9p21.3 region associated with heart disease are also associated with brain and abdominal aneurysms[8]. McPherson's group found the locus associated with arterial disease, suggesting that the region has something to do with the integrity of blood vessel walls. Nevertheless, figuring out the underlying mechanism is proving elusive for both groups. "We haven't found anything new through very thorough sequencing of the region," says Stefánsson. McPherson is looking for RNA sequences that might affect gene expression, but the region is long, she says, and even when the responsible sequence is ferreted out, the mechanism might not be obvious.

Even if the effect of an identified variant is tiny, the importance of finding a new mechanism could be huge, and genomewide association studies can uncover these in places in the genome that no one would think to look. What many researchers are looking forward to most is surprises. "For the past 20 years, traditional risk factors were the A-to-Z of cardiovascular biology," says cardiologist Heribert Schunkert at the University of Lübeck in Germany, who, along with Samani, confirmed the association of 9p21.3 with heart disease[3]. Many loci being identified today have no link to these risk factors. "That's exciting," he says. "It tells me we know very little about the true biology." ■

Monya Baker is the editor of *Nature Reports Stem Cells* and writes for *Nature* from San Francisco.

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*All Correspondence this week responds to Barbara Sahakian and Sharon Morein-Zamir's Commentary 'Professor's little helper' (*Nature *450, 1157–1159; 2007) and the related discussion at http://network.nature.com/forums/naturenewsandopinion.*

This week, Nature *launches an anonymous online survey to build on the informal questionnaire that the Commentary authors sent academics on the usage of brain-boosting drugs. In aggregate, the survey results will guide future editorial content on this topic. To take part, please visit: http://tinyurl.com/yq7nn3.*

The action of enhancers can lead to addiction

SIR — Sahakian and Morein-Zamir revive questions about the widespread availability and diversion of prescription medications for non-clinical use in healthy individuals (*Nature* **450**, 1157–1159; 2007). Such questions drove legislators to impose controls a few decades ago, when amphetamines and barbiturates were widely available.

Because the diversion of drugs is linked to their availability, the World Health Organization monitors their production and consumption by individual nations. In the United States, production of stimulant drugs has soared during the past two decades, and enough are now produced each year for the daily treatment of at least four million individuals. Even though stimulants and other cognitive enhancers are intended for legitimate clinical use, history predicts that greater availability will lead to an increase in diversion, misuse and abuse. Among high-school students, abuse of prescription medications is second only to cannabis use.

Although access to medications that improve our cognitive performance might be desirable in theory, these may have adverse medical consequences. Some limitations are necesssary, for these medications can be addictive. This is because cognitive enhancers such as the stimulants methylphenidate (Ritalin) and amphetamine amplify the activity of dopamine, a neurotransmitter that increases saliency, making cognitive tasks and everyday activities seem more interesting and rewarding. This learned experience can lead to abuse of the drug and to compulsive use and addiction in vulnerable people.

As we increase our knowledge of how the brain works, we may one day have safe interventions to improve cognition. In the meantime, we need to learn from history and avoid using them unnecessarily.

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Drugs can be used to treat more than disease

SIR — Your fine Commentary draws attention to some important questions (*Nature* **450**, 1157–1159; 2007). I agree with the point made by several commentators, that there is a need for better understanding of the long-term effects of using potential cognitive enhancers in an ecological setting. It is one thing to show a short-term positive effect on some artificial lab task; it is quite another to show that long-term use actually leads to sustainable performance gains on important real-world tasks, such as academic output. The former is easier to demonstrate, but the latter is what ultimately matters.

Unfortunately, progress on developing effective cognitive enhancers, and on understanding their long-term effects, is hampered by a shortage of focused research in this area. In general, the potential of enhancement medicine has yet to be fully appreciated.

Prevailing patterns of medical funding and regulation are organized around the concept of disease. Every pharmaceutical on the market with alleged cognitive-enhancing effects was developed as a treatment for some pathology. Its good effects on healthy adults' brains were discovered as fortuitous side effects. This disease-centred framework impedes the development of safe and effective enhancing medicines and has several consequences.

First, it makes funding hard to come by; it also makes it difficult to obtain regulatory approval for enhancement drugs. The result is that those who wish to research cognitive enhancement must often mask their work under the guise of addressing some 'respectable' disease.

Second, in order to gain access to the benefits of a cognitive enhancer, the user must first be classified as sick. This leads to the expansion of diagnostic categories and the invention of new pathological conditions — sometimes to cover cases that in earlier times would have been regarded as within normal human variation.

Third, it contributes to inequity in access. The main obstacle for someone who might be interested in trying modafinil or a related drug is not cost (which is similar to that of a large cup of coffee) but information: knowing that the drug exists and how to obtain it. This discriminates against people with little access to information.

With the cockcrow of enhancement medicine, we need to retool our regulatory paradigm. It is not only special occupations such as military commandos and air-traffic controllers that would benefit from good enhancement drugs. Other jobs are just as important and intellectually taxing — including the jobs of many scientists and academics. Anything that can help our brains deal better with the complex challenges of the twenty-first century is to be not only welcomed but actively sought. But it will require substantial investment to develop interventions that are both safe and effective in long-term use.

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Low dose of alertness drug counters 'family fatigue'

SIR — I have been taking 50 mg of modafinil almost daily for over a year. For most of my 74 years, I have struggled with fatigue and markedly reduced brain function every afternoon. This is part of my family history: my father and grandfather both structured afternoon naps into their schedules. I have found that caffeine and nicotine are either ineffective or cause a jittery nerviness. The side effects of the antidepressants I tried — desipramine, Paxil and Wellbutrin — were not worth the minimal benefits.

At first, I used modafinil only when I desired an extended high level of attention. Previously, I could work competently on the fracture-mechanics of high-silica stone (while replicating ancient tool-flaking techniques) for about an hour. With modafinil, I could continue for almost three hours. It did not make me 'smarter', but extended the length of concentrated focus.

When I used it on a three-day cross-country drive, I was not only more alert but found the journey more enjoyable and less tiring than previously.

I have not seen any data suggesting that modafinil is either habit-forming or easily abused (I have not looked for studies in

children). A 50-mg dose is quite low, but 100 mg does not increase the level or length of focus, for me at least, and can result in nervousness. As no 'high' is achieved, anyone taking too high a dose would soon cut down.

Competitive advantage is not a public-health issue at all, but a personal ethical and philosophical question. Today I will give my seven-year-old granddaughter a piano lesson, lead her in a chemistry experiment, listen to her sums and encourage her to enter any new words of her vocabulary into her personal dictionary. Am I trying to nurture her towards a 'competitive advantage'? You bet!

Charles Eaton
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Drugging unruly children is a method of social control

SIR — Sahakian and Morein-Zamir's reference to attention-deficit hyperactivity disorder (ADHD) as heritable and affecting 4–10% of children worldwide is contentious (*Nature* **450,** 1157–1159; 2007). The claimed incidence of ADHD varies strikingly over time: less than 0.1% in the United Kingdom before 1990, and now generally claimed to be between 1% and 5%. This variation is equally dramatic by country: highest in the United States, followed by Australia and Iceland, but low in Italy, for instance.

The diagnosis is in many cases questionable, and evidence for its heritability is shaky except in highly selected groups. The marked increase in the number of prescriptions of methylphenidate (Ritalin) — from 2,000 a year in 1991 to more than 300,000 in the United Kingdom today — says more about fashions in the diagnosis and treatment of naughty, inattentive or badly parented children than it does about a genuinely heritable 'disease.'

In the United States, the Federal Drug Administration has called attention to the 'epidemic' of schoolyard Ritalin use. As Sahakian and Morein-Zamir note, there is disturbing evidence of long-term, adverse sequelae associated with the use of such amphetamine-like drugs, especially on young and developing brains.

The assumption behind the cognitive-enhancer debate is that users are essentially making free choices about whether or not to take risks. But children being prescribed Ritalin are being drugged as a method of social control.

That, it seems to me, is a real ethical issue. If we don't recognize the real-world situation in which drugs are bought, prescribed and used, then the ethical debate is vacuous.

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Humans have always tried to improve their condition

SIR — The Commentary 'Professor's little helper' (*Nature* **450,** 1157–1159; 2007) entreats us to consider how the non-medical use of cognitive-enhancing drugs such as modafinil and Ritalin might influence society as a whole. They note concerns that a 'better, faster, stronger' mentality might coerce individuals into taking these drugs so that they can give themselves an edge.

Science and technology will continue to generate all sorts of new enhancers, and the quest for enhancement is not necessarily unfair or unethical. We humans are inveterate enhancers, striving to increase our intelligence and to improve our memory and powers of perception.

Consider spectacles: before they became commonplace, those who had good eyesight enjoyed an advantage over those who did not. Later, those who could afford spectacles joined those with naturally good eyesight — increasing (or decreasing?) natural unfairness. Enhancing technologies that improve eyesight are now widely available; we do not conclude that they are unethical because they are not globally accessible.

Before the invention of lamps or candles, most people went to bed at dusk; these inventions, and then electricity, enabled social life and work to continue into the night. Night owls can steal a march on their lazier or saner competitors, raising the bar and creating pressure for longer working hours. But such enhancement technologies are not considered unethical.

The same is and will continue to be true of cognitive enhancers. We must press for wider and more equitable access, turning our backs neither on technology nor on improving the human condition.

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Policy must recognize drug impact on different sectors

SIR — Sahakian and Morein-Zamir encourage us to explore a range of new issues raised by their reflections (*Nature* **450,** 1157–1159; 2007). In particular, we need to develop legal and social policies to guide the setting of parameters and milestones for integrating new enhancing technologies into healthcare for treatment — and into society for non-therapeutic application.

Policy-making is complex. It becomes even more so when the priorities of different healthcare systems come into play, which are inevitably influenced by the commercial interests of big-business pharmaceutical companies. One-size-fits-all policies will not work because of the range of multicultural factors that also need to be taken into consideration. For example, blanket regulation of cognitive enhancers will not play out evenly where socioeconomic status determines ease of access.

Sahakian and Morein-Zamir call for better drugs. Our call is for next-generation research and translation that is focused on regulatory policies. Those policies should recognize the differential impact of drugs on different segments of society. They should protect people from impulsive quick fixes and against vulnerabilities arising from short-sighted solutions.

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Rationality is a better basis for ethics than repugnance

SIR — Sahakian and Morein-Zamir's Commentary 'Professor's little helper' (*Nature* **450,** 1157–1159; 2007) makes an important contribution to the neuroethics of enhancement, as much for what it doesn't say as for what it does.

Much of the debate over neurocognitive enhancement has been guided by the so-called 'wisdom of repugnance'. We are encouraged to focus on our gut reaction to perfectly healthy individuals drugging themselves (or worse, their healthy children) for the sake of satisfying oversized ambitions. This highlights issues such as the need to earn one's success and self-esteem, and respect for our natural limitations.

Shouldn't we attempt a more rational analysis of the different contexts, methods and motives for neurocognitive enhancement and their likely outcomes, including the likely impact on society and human values?

Sahakian and Morein-Zamir provide a cautious yet open-minded assessment of risks and benefits, without any obeisance to the wisdom of repugnance. They have done us a service in framing the issues in this way.

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BOOKS & ARTS

Trinity says: Let's talk

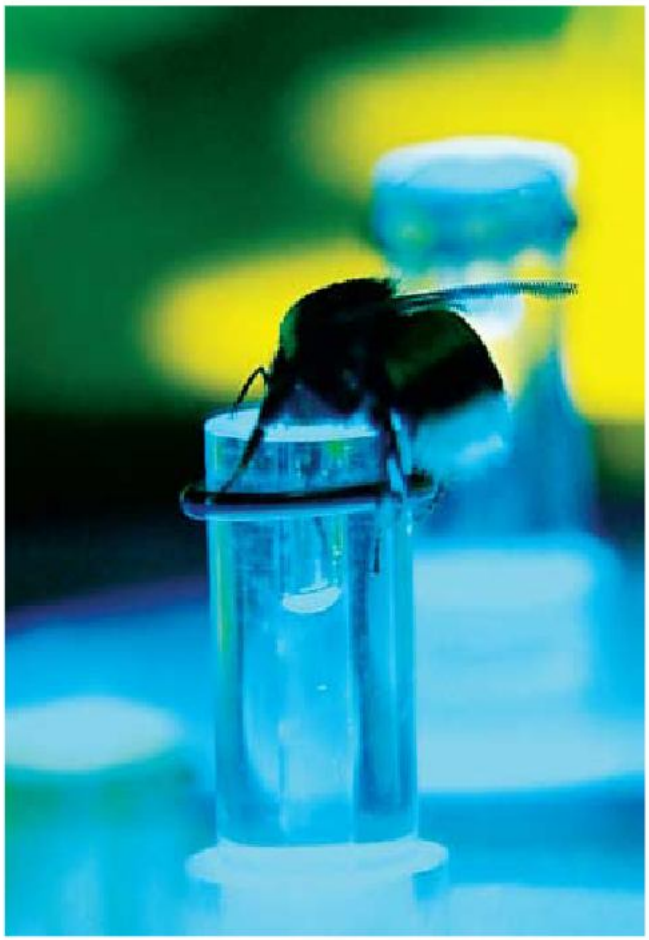
Dublin's new Science Gallery hopes to dissolve barriers between science and city through conversation. Director Michael John Gorman explains how the gentle art will bring new voices to research.

Science often erects barriers around itself. Consider the inscription over the entrance to Plato's Academy in ancient Athens: "Let no man ignorant of geometry enter here." Barriers to entry serve useful functions. They ring-fence institutions, centres of excellence and research projects. They allow groups of scientists to work on shared problems without having to continually question fundamental assumptions or waste time explaining their work to the uninitiated.

Yet rigid barriers can constrict those living on both sides. Interesting science is often created where boundaries are crossed, in border territories where connections are suddenly perceived between problems in seemingly unrelated areas. Critically for science's future, the widespread image of science as a narrowly focused, socially isolated vocation for the initiated can put off the brightest young talent from entering for one day, let alone decades. How can we make the world of science a little more inviting to those who may consider a whirlwind romance more thrilling than a life-long vocation?

Notoriously fickle, cynical and impossible to engage, young adults are considered a difficult audience by interactive science centres and museums. For this reason, many places focus their energies and marketing budgets on children and families. Some ambitious spaces such as the Dana Centre in London have tried to engage adult audiences with science by offering various series of events centred on controversial and newsworthy topics. The Wellcome Collection in London uses art to draw adults into thinking about biomedicine through its exhibition programme. The creative process itself is dissected by Le Laboratoire in Paris (see *Nature* **449**, 789; 2007) which nurtures 'artscience' collaborations in a space more like an artist's atelier than a science centre.

Conversation is the goal of the new Science Gallery, which opens on 2 February at Trinity College Dublin in the Republic of Ireland. This centre aims to capitalize on the university's scientific talent and location to design a new kind of interface between the university and the city. It is a territory where the business, cultural and policy communities can enter into direct creative and critical dialogue with researchers and young adult students.



Illuminating: the Science Gallery's opening exhibition tackles the science of light — here an exhibit explores vision in bees.

Rather than a repository for art works or science exhibits in cabinets, the Science Gallery is a place to talk. What happens when a nanotechnologist meets a fashion designer? When a biotechnologist meets a film-maker? When a 17-year-old meets a technology chief executive? Like the coffee houses of the seventeenth century, where one might have discussed the dissection of a dolphin with members of the Royal Society or encountered merchants and shipping agents swapping news, the Science Gallery hopes to be an informal, inclusive place for the exchange of ideas around emerging science and technology. Napkins have been designed for the café, on which visitors may sketch or jot down an idea. The generation of questions, open calls for ideas, public experiments and challenges is a key goal. Broad themes will be highlighted, ranging from fear to fashion to food. The Science Gallery opening features a series of installations and performances exploring the science of light, including lighting design and glowing textiles.

With numbers of graduates in science, engineering and technology declining in Ireland and elsewhere, the Science Gallery has a serious agenda to attract new pools of creative talent. Support has been forthcoming from the Irish government and companies such as Google, ICON, Dell and Wyeth.

So where might we go to spot promising scientists and engineers? In the Science Gallery, a range of activities will allow university researchers and members of the business and creative communities to mix with budding innovators. One is 'seed-dating', a variant on speed-dating designed to stimulate creative connections through rapid-fire two-minute conversations punctuated by a gong.

In common with other cultural centres, the ultimate challenge for the Science Gallery will be to keep its activities fresh and relevant, especially for a young audience for whom change is second nature. With the sole remit of killing off stale or unsuccessful programmes, the Science Gallery will appoint a 'jury of death', so resources can be redeployed. If the next generation of science centres is going to keep up with its audience, it will be critical to continue developing new and imaginative mechanisms to harness creativity, expertise and the desire for social interaction between communities. ■

Michael John Gorman is director of the Science Gallery, Trinity College Dublin, and author of *Buckminster Fuller: Designing for Mobility*.

The Science Gallery launches with Lightwave (2–9 February). Visit www.sciencegallery.com.

Van Allen remembered as belts turn 50

James Van Allen: The First Eight Billion Miles
by Abigail Foerstner
University of Iowa Press: 2007. 322 pp. $37.50

William E. Burrows

An iconic photograph marking the start of the space age shows three men thrusting a satellite above their heads. William Pickering, James Van Allen and Wernher von Braun lofted a scale model of the Explorer 1 satellite at the US National Academy of Sciences barely two hours after the real thing went into orbit on the night of 31 January 1958. It was the US response to the Soviet Union's provocative Sputnik launch months earlier.

Pickering headed the California Institute of Technology's Jet Propulsion Laboratory, which developed Explorer 1. Von Braun, a former Third Reich rocket prodigy who masterminded the V-2 missile, built the Jupiter-C launch rocket. Both engineers reacted to their feat with broad grins and irrepressible jubilation. The physicist Van Allen responded differently. His excitement was to come. Aboard Explorer 1 he had fixed a Geiger counter. It detected staccato cosmic-ray hits, which established that an enormous belt of radiation rings the Earth.

Van Allen "had a reputation as a practical physicist who applied the lessons of small-town Iowa ingenuity to outer space", writes biographer Abigail Foerstner. Her comprehensive and engaging portrait of Van Allen describes a tenacious individual whose modesty bordered on self-deprecation. For instance, he dubbed the phenomenal Explorer 1 mission "a 'shakedown' operation that succeeded on 'fool's luck'".

Others lauded Van Allen's scientific achievement. "The Russians sent up the first satellite but America made the first scientific discovery there, the most momentous discovery of the International Geophysical Year," according to Walter Sullivan, then the preeminent science writer of *The New York Times*. At a conference in Europe soon after, the physicist Robert Jastrow used the term Van Allen radiation belt for the first time and the name soon stuck. A permanent new landmark in the heavens, and a hazardous region to be traversed by future spacecraft, the Van Allen belts comprise giant lobes of charged particles trapped by Earth's magnetic field that extend thousands of kilometres into space.

Foerstner, a former science writer for the *Chicago Tribune*, trains a journalist's eye on her subject. She began interviewing Van Allen in his office at the University of Iowa in 1998, and on until his death in 2006 at the age of 91.

A model of Explorer 1 held by (from left) William Pickering, James Van Allen and Wernher von Braun.

COLLECTION JAMES VAN ALLEN

A sign on his door gave its exact longitude and latitude, reflecting the dry humour of an individual whose scientific competence never overshadowed his human qualities. To her credit, Foerstner portrays a complete human being, not a scientific automaton, with richly detailed references to his wife Abigail, his children, other relatives, adoring students, and other rocket scientists. One photograph shows Van Allen whimsically holding up a T-shirt proclaiming: "ACTUALLY, I AM A ROCKET SCIENTIST."

> "Van Allen's achievements as a space pioneer far surpass the discovery of the radiation belt named after him."

Like Pickering and von Braun, Van Allen was passionately preoccupied with unlocking secrets in the then-new realm of space. Von Braun's V-2s, which terrified the residents of wartime London and Antwerp, were a gift to Van Allen, enabling his radiation detectors to pierce "the cosmic ray ceiling of the atmosphere", as he put it.

The drive to get his instruments into space with little or no budget forced Van Allen to be inventive. He initially flew his instruments on weather balloons, then created the smaller, cheaper Aerobee research rocket to replace the V-2, and eventually launched small rockets from navy balloons to reach even higher; he christened these 'rockoons'.

Van Allen's achievements as a space pioneer far surpass the discovery of the radiation belt named after him. Nevertheless, the Explorer 1 mission that helped lay the foundation for space science proved to be a hard act to follow and obscured many of his later accomplishments that reached well beyond Earth.

After being carried on a series of Earth-orbiting science satellites called Injun, his clever instruments next flew to Venus on Mariner 2, and then across the Solar System aboard Pioneer 10. His guiding presence accompanied Voyager 2 when it flew its 12-year grand tour of the four outer giant planets that ended at Neptune in 1989.

As Foerstner concludes: "His instruments on board more than 200 rockets, satellites and space probes transmitted data over six decades. Pioneer 10 alone, launched for a 21-month mission in 1972, sent Van Allen more than 30 years of readings that helped us recognize that the boundary of the Solar System extended billions of miles past Pluto." A journey indeed. ■

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IMPERIAL WAR MUSEUM/THE ART ARCHIVE

The Food Queue (1918) by C. R. W. Nevinson depicts food rationing in First World War Britain.

Starvation: crime and punishment

Hunger: A Modern History
by James Vernon
Harvard University Press: 2007. 384 pp. $29.95, £19.95

Michael Sargent

For any nation, eliminating the risk of serious malnutrition or starvation is a mark of developmental maturity. Getting there requires a combination of factors: acute scientific and political awareness, appropriate institutions and a benign economic situation. James Vernon charts progress towards this goal in Britain, between the 'hungry forties' (1840s) and the emergence of its welfare state a century later.

Britain was the first country to industrialize, moving towards dependence on imported food, truly representative government and proficient public services. Other European countries had similar trajectories but took longer as they were gripped by more rural poverty and industrialized less aggressively. The land-rich United States and Australia had low population densities and swallowed up their 'huddled masses' efficiently. Yet even the United States could not entirely avoid malnutrition; in the 1900s, niacin deficiency (pellagra) was extremely common in the South.

Vernon's story begins in an era of unprecedented economic development and extraordinary pressure on society's poorest strata. The oracle of the age, Thomas Malthus, saw hunger as God-given discipline for the profligate and the reproductively overenthusiastic. His thinking underpinned the New Poor Law of 1834, a measure designed to make poverty feel like a crime, that involved incarcerating paupers in the workhouses immortalized in Charles Dickens' *Oliver Twist*. Deeply critical, *The Times* newspaper tried to stir polite society's conscience with lurid reports of the fate of victims of the 'Starvation Act', malthusianism and the Corn Laws (that banned cheap imports of grain in years of scarcity to protect the interests of wealthy landowners).

Devastating famine in Ireland brought by the potato blight in 1845–47 provoked neither sympathy nor much governmental assistance for these British subjects. To Charles Trevelyan (a former pupil of Malthus), with some responsibility for dealing with the crisis, it was "an act of providence"; "a sharp and effectual remedy" for the over-population considered an obstacle to land reform. Later in the century, the intense distress conveyed by photographs and reports of Indian famines made the moralizing face of malthusianism seem shameful and the Imperial management look incompetent.

After the turn of the century, research on the scientific basis of nutrition gained momentum and started to shape governmental thinking. Seebohm Rowntree's dietary survey of 1904 showed that the daily calorie intake of 30% of the population of York was less than the minimum required for a healthy life. In the British army, two-thirds of volunteers for service in the Boer War failed to meet physical-fitness requirements because they were undernourished. During the First World War, scientists were recruited to devise diets for soldiers and civilians and to implement food rationing.

Knowledge of the health benefits of vitamins and minerals, gained in the 1920s, raised the question of who should guide the public towards better dietary habits. The problem was severe: surveys in the 1930s revealed some malnutrition in half of the British population, not only the poor. With evidence accumulating that infant mortality, stunting of growth, susceptibility to chronic disease and work-time lost through sickness or lassitude could all have nutritional causes, public policy had to take new directions. One of the most important schemes introduced nutritionally sound meals in school. More problematic was the attitude of the public, often deaf to official advice yet responsive to an advertising industry whose influence was not always good.

Starvation emerged as a potent political weapon in the twentieth century. Hunger strikes by suffragettes, the Irish and Indian nationalists embarrassed policy-makers in ways that would have cut no ice with early nineteenth-century governments. Hunger marches before the First World War and in the 1930s, staged as protests over unemployment and the dole, had consequences that emerged only after 1945. They symbolized a great wrong inflicted on the working classes that would be put right only by a welfare scheme that secured minimum living standards.

Hunger is a thought-provoking book. Sharply

EXHIBITION

Ancient orders of nature

Martin Kemp

The blind giant, Orion, his left arm extended in a precautionary manner, proceeds along a road in a sumptuous landscape. He is guided by his companion, Cedalion, who stands on his shoulders and directs him towards the rising Sun, prescribed as a cure for his sightless condition.

Orion had been blinded in Chios by the father of Princess Merope, whom he had vilely attempted to rape. The goddess Diana coolly observes Orion's uncertain progress from the cluster of clouds that still veil the giant's head from the restorative Sun. It was Diana who later translated the giant into the starry constellation, after Orion had unwisely directed his rapacious intentions towards her.

Even if the subject of the blind Orion was very rare, we are familiar enough with this kind of picture from the Renaissance and Baroque eras. The *Landscape with Blind Orion Searching for the Sun* (pictured) was painted in 1658 by Nicolas Poussin, the French artist resident in Rome during the last years of his life.

Like a number of paintings on ancient themes, it was inspired by an account of a lost work by an ancient author. Lucian, in *The Hall*, describes a sequence of wall paintings: "The next picture deals with the ancient story of Orion. He is blind, and on his shoulder carries Cedalion, who directs the sightless eyes towards the East. The rising Sun heals his infirmity."

Beyond the highly controlled naturalism, there is little to suggest that either the subject or the artist might be engaged with the scientific culture of the time. However, as Ernst Gombrich showed in 1994, Poussin's most direct literary source was a reference book on classical mythology that aspired to demonstrate how "all the doctrines of Natural and Moral Philosophy were contained in the fables of the ancients". It was published in 1551 in *Mythologiae* by Natalis Comes (Natale Conti), who went to acrobatic lengths to show how ancient myths embodied modern discoveries in the guise of allegory.

Poussin, as the supreme "philosophical painter" of his (or virtually any other) generation was naturally drawn to a source that promised to bind the wisdom of his revered 'ancients' to the new sciences. Poussin, who moved in high intellectual circles in Rome, insisted on the rational basis of art as visual knowledge.

He defined the proper role of painting as a form of rational scrutiny, distinct from mere seeing and passive imitation. It was to reveal in form and content the underlying order of the created world and the integral position of humans in the divine system of nature. The landscape is based on the wonderfully fresh studies that Poussin made directly from nature, but the landscape's naturalism is reshaped in terms of what he called "the order and the mode and the species of things".

But what of the specific interpretation of the Orion myth provided by Conti? In summary, Conti's horribly tangled 'scientific' reading runs as follows. Improbably born of the triple copulation of Jupiter, Neptune and Apollo with the hide of oxen, Orion was accordingly composed of air, water and fire, as expressed as wind, rain and thunder. Orion's journey to Chios, his rape of — in Conti's version — Aerope (air) and blinding signify the diffusion of his watery powers in the form of vapours, which rise impotently into the upper atmosphere. The ascending vapours are gathered by the cold power of the Moon (Diana) to be cast Earthwards as rainstorms. Conti concludes the story represents the "cyclical and mutual generation and destruction of the elements".

Conti's musings have long been abandoned. Poussin's painting, on the other hand, continues to breathe a timeless sense of the inherent grandeur of nature stirring with inner life and cyclical renewal. Like Cedalion, Poussin saw himself standing on the shoulders of both the giants of antiquity and of his own time, to see further into the truths of the created world. ■

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***Poussin and Nature: Arcadian Visions* runs 12 February–11 May at New York City's Metropolitan Museum of Art.**

METROPOLITAN MUSEUM OF ART, FLETCHER FUND, 1924 (24.451)

focused and tightly argued, it excludes a few peripheral topics. One wonders why Vernon chose not to explore the evolution of British diets in Victorian times. Sugar-sweetened tea, bread-and-jam and "sugar butties" were adaptations by the poor to industrial employment that alleviated hunger cheaply but also created malnutrition. More curious is the omission of the improvement in stature and life expectancy that started with the repeal of the Corn Laws. The importance of journalism in raising consciousness of hunger as a social problem is discussed, but less emphasis placed on writers such as Dickens, Elizabeth Gaskell and Henry Mayhew. The attitude of the Church is also neglected.

Vernon's story ends in the 1940s when many newly independent nations took responsibility for huge populations. Destitute on a scale never seen in Europe, they faced an accelerated birth rate if the food supply improved. Severe famines have occurred and have been blamed on poor economic management and distribution rather than Malthus' prophecy of moral punishment. Malnutrition is a more insidious problem. In the developing world, vast numbers of people are deficient in vitamins and micronutrients because the range and volume of their food intake is minimal. Consequences include stunted growth of children, low birth weight, decreased immunity to infection and damaged eyesight. Although appropriate welfare schemes can be devised, the solution may lie ultimately in increased economic prosperity. There is little doubt that when incomes improve, the quality of food intake improves. ■

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Hidden treasures

In the first of a monthly series on small museums, **Alison Abbott** profiles the University History Museum in Pavia, which recalls the key role of northern Italy in Enlightenment science.

Antonio Scarpa (1752–1832) was by all accounts a tyrant. But the best legacies are often left by those who are hated or feared by their colleagues. Scarpa's legacy is a marvellous suite of anatomical preparations. Dried and browning or bobbing in preservation fluid, they are held by the University History Museum in Pavia, Italy. Scarpa's own pickled, dismembered head watches over from a high alcove, nearly two centuries after his death.

Scarpa's cache, extended during the nineteenth century, is just part of the museum's remarkable and diverse collections, including several centred on other famous Pavian professors, such as Alessandro Volta and Camillo Golgi. Together, these holdings represent some of the major turning points in the history of science and illustrate the key role that northern Italy played in those times.

Pavia became a major European centre of science after the 1770s. Empress Maria Theresa and her son and successor Joseph II imposed Enlightenment 'Education and Science Plans' on this distant outpost of their Austro-Hungarian empire, in the region of Lombardy. The plans insisted on experimental scientific method, and advised that teaching and research should be carried out by "masters of proven merit or those with great promise".

Many Pavia professors left their name to science in the subsequent two centuries — think of Volta's volt, Golgi bodies in the cell, or the drug scopolamine named after the naturalist Giovanni Scopoli. Scarpa himself left Scarpa's ganglion (in the brain), Scarpa's triangle (in the thigh) and a record nine further eponyms.

Some professors also left their body parts, willingly or not. In addition to Scarpa's head, the museum displays his kidneys and four of his fingers, eerily swollen in their preparation fluid, their skin starkly whitened, their nails blackened. The aneurism that killed mathematician Vincenzo Brunacci in 1818 sits nearby. The bladder of the influential naturalist Lazzaro Spallanzani, who died of kidney cancer in 1799, is probably uncomfortably close, given that Scarpa hated him beyond reason. A dozen or so bony skulls of professors and other local heroes line up on a high shelf, among them a plaster cast of Volta's unusually large skull. These were probably used by nineteenth-century phrenologists.

These grotesqueries were far from gratuitous. They were assembled in the noble service of morbid anatomy, a new science, introduced by the Paduan Giovanni Morgagni (1682–1771), which related for the first time symptoms of a disease to the state of an internal organ. A yellow skin, for example, could be revealed on autopsy to be associated with a degenerated liver. Scarpa was Morgagni's favourite pupil.

SISTEMA MUSEALE DI ATENEO, PAVIA

Pavia's frescoed anatomy theatre: built for Antonio Scarpa to demonstrate dissection.

Most of the anatomical preparations in the collection were dissected at autopsy by Scarpa and his pupils from ordinary patients passing through Pavia's reputed San Matteo hospital. Each had something to demonstrate about a particular disease. They provided a base for another new science — physiology — and guided surgeons wanting to intervene ever more subtly in the workings of the body.

A collection of skeletons includes a sobering number from post-natal deaths. These, and a few wax anatomical models in the voluptuous Florentine style, were also used for teaching, which Scarpa took very seriously. When he took up his chair in Pavia in 1783, Scarpa ordered the construction of a modern anatomy theatre to demonstrate through dissection. This architectural jewel, decorated with frescoed angels holding aloft the silver and ivory surgical instruments that Joseph II donated in 1786, is now part of the museum.

Golgi, who won a 1906 Nobel prize for physiology or medicine, and Volta — whom Napoleon made a count in 1810 — have a room each in this colonnaded eighteenth-century museum. Golgi's microscopic preparations, instruments, drawings and other memorabilia tell the story of his many scientific achievements, including the 'black reaction', a stain that allowed researchers to see individual nerves under the microscope for the first time. In Volta's room are 150 or so intriguing pieces of apparatus, which he used or invented. The museum's collection of historical physics instruments was extended to more than 800 items in preparation for the 1999 bicentenary of Volta's invention of the electric battery.

Volta, like Scarpa, also had a lecture theatre built for his sole use. With its frescos depicting the physicist's instruments and experiments, it is another architectural masterpiece — and is still used for special lectures today. Volta, not being a direct rival, was someone Scarpa actually considered to be a friend. ■

Alison Abbott is Nature's senior European correspondent.

The University History Museum in Pavia, Italy is open to the public on Mondays, Wednesdays and Fridays, and by appointment. Visit http://tinyurl.com/36dzqb.

NEWS & VIEWS

SEX DETERMINATION

Some like it hot (and some don't)

David Crews and James J. Bull

There is a widely accepted theoretical explanation for why sex in some species is determined at the embryo stage by environmental factors such as temperature. That theory is now supported by experiment.

How the sex of offspring is determined seems simple enough if you don't look beyond ourselves. For humans, the system is genotypic: two X chromosomes, and you're female; an X and a Y, and you're male. There are plenty of variants of this system, but in many reptiles an entirely different mechanism applies: sex is determined by the temperature of the incubating egg, and clutches can be all-male, all-female or somewhere in between.

On page 566 of this issue[1], Warner and Shine answer a long-standing question about the evolutionary significance of sex-determining mechanisms in reptiles. Four decades ago, it was reported[2] that incubation temperature determined sex in the African red-headed rock lizard, an observation that seemed to fly in the face of evidence for sex chromosomes in several other reptiles[3]. Scientists soon realized that both types of sex-determining system were not only widespread in reptiles but also highly developed, and at the time they seemed to be mutually exclusive[4]. What has remained a puzzle is whether there is an adaptive benefit of temperature-dependent sex determination (TSD) and how that benefit might work. Using an Australian lizard, Warner and Shine find the long-sought evidence of an adaptive benefit of TSD.

D.GARNETT

Figure 1 | The jacky dragon, *Amphibolurus muricatus*. This is a species of reptile in which the sex of the offspring is determined by the egg-incubation temperature, and was the experimental model chosen by Warner and Shine[1]. The jacky dragon's comparatively short lifespan of some 3–4 years makes it especially suitable for sex-determination research.

The main model suggested to explain the advantage of TSD, or of any sex determination in response to an environmental cue, takes an idea from Trivers and Willard[5]. This posits that in some circumstances a species will have greater reproductive fitness if the offspring are male instead of female, whereas in other circumstances the reverse is true. For example, if there is a premium on large size for male reproduction, an offspring deprived of food such that it will be born small and remain smaller than average throughout its life may have higher fitness as a female than as a male. From there, the argument for why sex should be environmentally determined is merely that, if there is a strong benefit to controlling offspring sex ratio to suit the circumstances, natural selection will favour a mechanism to do so[6].

In reptiles, sex is determined during the embryonic stage. For environmental sex determination to fit this model, something happening to the egg or embryo must carry over into adult fitness, and the effect must be one that works differently for males than females, so mere survival to hatching does not provide an explanation. The puzzle is that, because so much growth happens between hatching and maturity in a reptile, it would seem that all effects of embryonic temperature would be erased by adulthood.

There has been no shortage of ideas for how this model[6] could apply to reptiles, from supposing any of several direct effects of incubation temperature on egg-to-adult fitness, to allowing mothers to manipulate offspring sex ratio by choice of nest site[7]. However, until now all explanations have relied on inferences about fitness effects, not measurements.

By integrating several techniques for lab and field studies, and with a careful choice of study organism, Warner and Shine[1] show that incubation temperature affects lifetime fitness and does so differently for males and females. Their study organism was a short-lived lizard, the jacky dragon (Fig. 1). The use of a short-lived species is important because the differential effects of incubation temperature are expected to be strong in short-lived species, and not necessarily so in long-lived ones.

The next trick required a way of producing both sexes across a wide range of incubation temperatures. The theory holds that only the sex of relatively higher fitness should be produced at any one temperature, and indeed, TSD is often so extreme that only one sex is produced across a wide range of incubation temperatures. Thus a test of the theory requires producing both sexes at temperatures where one sex is normally absent. Male jacky dragons are produced in only a narrow, intermediate temperature range. So to produce males at high and low temperatures, Warner and Shine used the now-common method of applying chemicals to the egg that interfere

with steroid hormone biosynthesis, in this instance the aromatase inhibitor fadrozole[8].

Eggs were incubated in the lab at one of three temperatures (low, intermediate, warm), and the hatchlings were released into outdoor enclosures (about 30 lizards in each of 6 enclosures). The lizards were allowed to grow up, mate and produce offspring in these enclosures over a period of 3.5 years. To measure fitness — reproductive success — Warner and Shine established parentage of each of the offspring by genotyping DNA microsatellites. All offspring born in the enclosures were unambiguously assigned to specific parents, thus bypassing any indirect measures of presumed mating success and fecundity.

Lifetime reproductive success showed some surprises. For females, it was expected, first, that warmer incubation temperatures would lead to larger body size (because warmer temperatures lead to earlier hatching); and, second, that body size would correlate strongly with fecundity. Thus female fecundity should increase with incubation temperature. This compound expectation was only partly supported: female lifetime fitness was highest at the warmest temperature, but no appreciable fitness difference was found between the intermediate and low temperatures. For males, there was no obvious basis for prediction, but males from intermediate temperatures had appreciably higher fitness than males from low and warm extremes. In all, the fitness measures matched the theory, but most of the fitness effects of temperature defied intuition.

The study[1] provides directions for future work. The most important concerns the mechanistic bases by which incubation temperature affects male versus female fitness. There is accumulating evidence that incubation temperature in TSD lizards has a variety of behavioural, anatomical and physiological effects, including directly acting on brain development[9,10]. In addition, even though offspring are either male or female in terms of their gonads, hormone levels throughout life vary according to the individual's incubation temperature, further contributing to a gradation of attributes that translate into fitness differences within a sex caused by incubation temperature. To the extent that such interactions exist, TSD may have evolved to be somewhat self-reinforcing, in essence providing the basis for much of its own benefit. It will thus be interesting to solve the mechanistic link between temperature and fitness, to augment the observations that Warner and Shine have at last provided to resolve the riddle of reptilian sex determination.

There is also a wider picture to this line of research. It has been suggested that sex determination by temperature or other environmental factors is ancestral to genotypic sex determination, and that elements of TSD can be found in mammals[11]. Even in humans, conditions during gestation have lasting effects throughout life, with recent work indicating a connection with coronary disease, obesity, diabetes, cancer, cognitive dysfunction and infertility[12]. ■

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NANOMATERIALS

Golden handshake

John C. Crocker

Three-dimensional nanoparticle arrays are likely to be the foundation of future optical and electronic materials. A promising way to assemble them is through the transient pairings of complementary DNA strands.

One of the staple concepts of nanotechnology is that of 'growing' useful materials or devices by coaxing a random mixture of microscopic parts to assemble spontaneously into a desired structure. Versatile self-assembly schemes have been demonstrated that use DNA as the primary building material[1]. In this issue, two research teams, one led by Oleg Gang (Nykypanchuk *et al.*, page 549)[2] and the other by Chad Mirkin (Park *et al.*, page 553)[3], recount how they have built on the successes with DNA to aid the self-assembly of gold nanoparticles. Their technique should also work for other varieties of technologically exciting nanoparticles.

Progress in achieving the directed self-assembly of nanoparticles had been elusive, owing to one potentially daunting requirement: selective adhesion. Each microscopic part must be engineered so that it sticks only to the others it should abut in the desired final structure. In earlier experiments[4], nanoparticles were found to form ordered arrangements when a surrounding solvent was evaporated. In this case, however, the final structures depended sensitively on the particle chemistry and charge.

This is where DNA comes into its own. Particles carrying complementary strands of DNA selectively adhere to each other when the strands 'hybridize' to form the familiar DNA double helix. The final architecture is thus determined not by chemistry or charge, but by the lengths and nucleotide sequences of the DNA strands. That promises a versatile assembly scheme that might be used with particles of nearly any material to fabricate nanocomposites or 'metamaterials'[5] with unusual electronic and optical properties. The applications of such materials might include high-efficiency solar panels and lasers, super-resolution microscopes — and even coatings to render objects invisible.

Nykypanchuk *et al.*[2] and Park *et al.*[3] both start by grafting DNA to gold spheres of the order of 10 nanometres in diameter to give two populations of DNA-capped particles, A and B. Each sphere bears several dozen strands, and the ends of the strands on A-type and B-type particles are complementary. This configuration means that spheres of one type will selectively adhere to spheres of the other, but neither type of sphere will adhere to its own kind.

The authors mixed the A and B spheres in water. Under the right conditions, they found that the nanospheres were rapidly guided, as the DNA strands hybridized, to arrange themselves into well-ordered arrays. The resulting crystal had body-centred-cubic crystal symmetry, with A and B spheres taking up alternating locations in the lattice, so that each A sphere was surrounded by eight B spheres and vice versa (Fig. 1). Such a structure — known as a CsCl lattice after crystals of caesium chloride, which take the exact same form — provides the maximum possible number of A–B adhesion contacts.

Both Nykypanchuk *et al.*[2] and Park *et al.*[3] report that crystallization requires the DNA-binding regions to be connected to the gold spheres by flexible spacers, also made of DNA, that are roughly as long as the sphere diameter. Moreover, crystallization happens only at higher temperatures, at which the DNA binding strands are dynamic, continuously forming double helices and dissociating back into single strands.

The DNA in these experiments is being used in a fundamentally different way from its use in earlier DNA self-assembly techniques such as Ned Seeman's 'DNA tile' approach[1]. There, each constituent tile of the structure was made of interconnected DNA double strands. Each tile had one binding strand dangling from each corner, so that it could mate with neighbouring tiles. The structure of each tile was thus controlled at the molecular scale. The chemical process for attaching DNA strands to nanoparticles[2,3], by contrast, is essentially random,

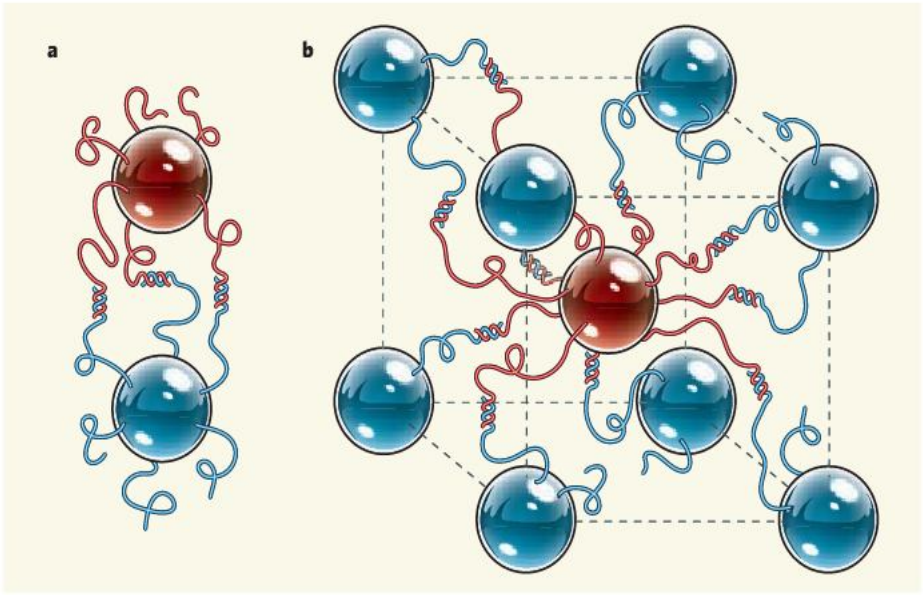

Figure 1 | Self-assembly through DNA strands. **a**, Nykypanchuk *et al.*[2] and Park *et al.*[3] mix two populations of nanoscale gold spheres, A (red) and B (blue), which have long DNA strands covalently grafted onto their surfaces. The ends of these strands contain complementary sequences. When A and B are close together, the ends hybridize into a double helix, forming bridges that pull the spheres together. **b**, Under conditions in which the bridges can form and dissociate dynamically, the spheres self-assemble into large, ordered 'CsCl' arrays with a body-centred-cubic symmetry; one unit cell is shown.

and scatters DNA strands over the gold sphere's surface, rather than at just eight nearest-neighbour locations. The exact number of strands varies from sphere to sphere.

Remarkably, ordered arrangements of the nanoparticles can form despite these random variations in their individual structure. The long spacers and the dynamic binding process seem to be crucial. Flexible spacers fluctuating in space ensure that an extended spherical cloud of strands surrounds each core, washing out the random pattern in which the strands are anchored to the particles. When the clouds of complementary neighbouring particles overlap, hybridization forms transient DNA bridges that briefly pull pairs of spheres towards each other.

In essence, the nanoparticles reach out to each other using their long spacer arms, and temporarily 'shake hands' with their complementary DNA strands. The net attractive interaction is proportional to the time-averaged number of bridges between a pair of spheres. This in turn is determined by the degree of overlap between the two DNA clouds[6].

Unlike the strictly determined binding that drives other DNA-based assembly techniques, suspensions of nanoparticles with such handshaking interactions mimic[7] the phase behaviour of atomic materials, but using engineered interactions. The ordered arrangement of A and B spheres, for example, mirrors the alternating positive and negative ions in a salt crystal, which also have a long-range, spherically symmetrical attraction.

The analogy is not perfect: Park *et al.*[3] report that one of their samples forms a face-centred-cubic, rather than a body-centred-cubic CsCl structure, when incubated at higher temperatures. They argue that this behaviour stems from a competition between the contributions to the system's total free energy of sphere entropy (which favours the more densely packed face-centred-cubic structure) and A–B binding energy (maximized by the CsCl structure).

Realizing the potential of these new materials will certainly require more research to stabilize their structure. The long DNA spacers imply that the resulting nanoparticle array is roughly 90% water, and is probably quite fragile. Still, existing techniques can probably be adapted to fill the gaps with gels or solid ceramic to yield a robust, solid material. Better models of the handshaking interaction will also need to be developed, validated and applied to computing what periodic structure a given DNA sequence will produce.

Even more exciting would be the possibility of attaching DNA to non-spherical nanoparticles — perhaps preferentially to different crystal facets — to create directional bonding and more complex structures. The ultimate dream is the creation of a DNA tool-kit that will make possible the self-assembly of nearly any material reliably at the nanoscale. ■

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50 YEARS AGO

The seriousness of such neglect [of the problem of "cultural erosion"] is unmistakable to any thoughtful reader of Mr. Hoggart's book ["The Uses of Literacy"]... Writing with deep feeling and imaginative insight, Mr. Hoggart seeks to show that the under-educated in Britain — the three-quarters of the population whose schooling now ends at fifteen and whose distinguishing characteristic is rather lack of education than of money — are changing their traditional ways, and not for the better. Their freedom from poverty has exposed them to new and very deleterious influences, and while the gross prejudices and appetites are deliberately and even scientifically stimulated, the needs of the more serious-minded among them are neglected.
From *Nature* 1 February 1958.

100 YEARS AGO

The Prolongation of Life. By Elie Metchnikoff — Most people desire to live long, and hence Prof. Metchnikoff's book is sure to have many readers. He not only discusses the means by which life may be prolonged, but he also examines the question whether it is desirable to prolong it... Prof. Metchnikoff is of opinion that when old age approaches, the phagocytes, which have hitherto been man's friends, become his enemies, and hasten death by devouring the essential cells of the vital organs of the body, especially those of the nervous system. These cells are rendered particularly vulnerable to phagocytes by the action of poisons manufactured by the bacteria of the large intestine, and Prof. Metchnikoff suggests that this might to a large extent be prevented by taking skimmed milk which has been boiled and rapidly cooled, and on which pure cultures of the Bulgarian bacillus have been sown. This produces a pleasant, sour, curdled milk containing about 10 grams of lactic acid per litre, the lactic acid of which prevents intestinal putrefaction.
From *Nature* 30 January 1908.

50 & 100 YEARS AGO

CELL BIOLOGY

Dying to hold you

Kimon Doukoumetzidis and Michael O. Hengartner

Certain cells bind so tightly to each other that, on occasion, one cell ends up inside another, usually with fatal consequences for the ingested cell. This involuntary cell death might help protect us from cancer.

The epithelial cells that cover most of the surfaces of our bodies create tight physical barriers that protect us from the outside world. To do this effectively, these cells need to stick to each other very well — which they do, thanks to molecular Velcro proteins known as cadherins. In a provocative study published in *Cell*, Overholtzer *et al.*[1] show that unless epithelial cells are physically restrained, their strong affinity for each other can turn into a deadly embrace, with one cell ending up inside the other. To make the story even more intriguing, the authors show that the internalized cell usually dies by means of a molecular mechanism unlike any other known.

Normally, epithelial cells sit on an extracellular matrix — a complex meshwork of molecules that acts as a support net for cells to crawl on and attach to. Interaction with this matrix is essential not only for epithelial-cell function, but also for these cells' very survival; epithelial cells that detach from the extracellular matrix rapidly activate a cell-suicide programme known as apoptosis, which in animals leads to the elimination of cells that are in excess, in the wrong place or potentially dangerous[2–5].

Once a cell decides to die, it is rapidly recognized by specific 'eat-me' signals on its surface, taken up and degraded by a neighbour[6]. This clearance process is by necessity highly selective; living cells lack such markers and are left alone. There are, however, some exceptions to this rule. For example, pathologists have often reported the occurrence in cancer tissues of 'cannibalistic' cells, which can apparently ingest other normal-looking cells[7].

What could be the basis for this odd behaviour? Overholtzer and colleagues[1] propose an attractive answer. They report that, when mammary epithelial cells are separated from the extracellular matrix and left to float in a suspension, they frequently form 'cell-within-cell' structures, whereby one epithelial cell is either partly or completely inside another.

Surprisingly, the cell-within-cell phenomenon the authors observed is not triggered by apoptosis. First, many of the ingested cells looked to be alive and well, and those that were dead had clearly died from something other than apoptosis. Second, the internalized cells did not present the eat-me signals typical of apoptotic cells. Rather, the internalization process seems to depend on the cadherin system, and the authors suggest that ingestion is the result of a tragic mistake, a friendly hug gone awry.

When epithelial cells meet, because of the strong self-adherence properties of cadherins, they will rapidly try to maximize their surface interactions[8,9]. Normally, the extent of such cadherin-mediated interaction is kept in check by the cells' attachment to the extracellular matrix. The resulting balance of forces leads to the formation of a neat cobblestone pattern characteristic of epithelial sheets (Fig. 1a).

In the absence of solid support, however, there is little to oppose cadherin-based self-adhesion. Consequently, epithelial cells' urge to maximize their surface interactions can be pushed to its logical extreme, whereby, over the course of a few hours, one of the two cells actively 'covers itself' with the other. The topological consequence is that the overeager cell ultimately finds itself, probably quite unwittingly, fully inside its partner (Fig. 1b). The authors name this unusual, unexpected internalization process entosis, from the Greek *entos*, meaning inside or within.

That entosis is neither premeditated murder nor suicide is further supported by Overholtzer and colleagues' observation of what happens immediately after internalization — namely, not much. Unlike apoptotic cells, which are rapidly degraded after being engulfed, the internalized cell — let's call it an entocyte — lives on inside its host, blissfully ignorant of its precarious condition. The host cell, in turn, also seems to have little idea what to do with its guest, or perhaps even that it is hosting one. Neither side is fully committed to any further step — indeed, the internalization process is reversible, and a small fraction of entocytes are eventually released back into the 'wild' of the Petri dish, with no apparent long-term damage.

For most entocytes, however, the story has a tragic ending. At some point, often many hours after internalization, the ingesting cell suddenly switches from gentle host to ogre, and kills its guest entocyte. This 'entocide' uses a novel, crude, but effective strategy. The host simply fuses the entocyte with its lysosomes — acidic bags of digestive enzymes used by cells to degrade and recycle large molecules or, in this case, the unsuspecting guest. And the entocyte is literally digested alive. It is not currently known what causes this switch in behaviour.

These observations are spectacular, but many sceptics will question their physiological relevance. Are there ever free-floating epithelial cells in our bodies? As mentioned above, cell-in-cell structures can often be detected, admittedly at low frequency, in various cancers. But whether these are the result of self-adhesion-mediated entosis or some other mechanism is not known.

And what about possible functions? Clearly, a mechanism such as entosis could help eliminate mislocalized epithelial cells. Such situations might arise during development, or during cancer progression. Indeed, Overholtzer *et al.* posit that entosis functions as a barrier to tumour formation by eliminating metastatic cancer cells that have escaped from their physiological niche. But one could also imagine the opposite. Could cancer cells take advantage of entosis, and transiently crawl into their healthy neighbours to escape immune surveillance or chemotherapy?

Finally, it will be of great interest to determine how widespread entosis is in other species, and to identify genes involved in this process. After apoptosis, necrosis and autophagy, entosis is not only the latest addition to the Greek-derived, cell-death-associated jargon,

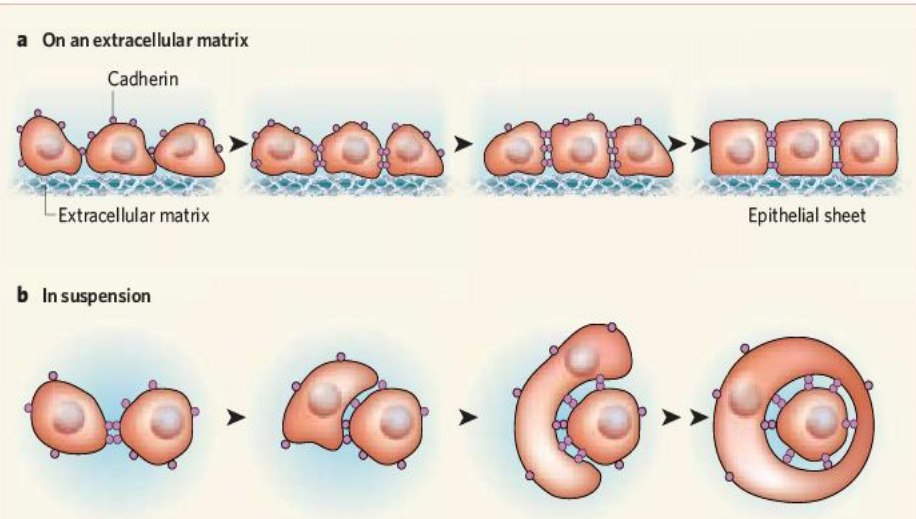

Figure 1 | Breaching the limit of intimacy. **a**, For epithelial cells attached to an extracellular matrix, maximum surface interaction through cadherins leads to well-ordered epithelial sheets. **b**, But in the case of epithelial cells in suspension, such as those studied by Overholtzer and colleagues[1], increased interaction between surfaces can lead to complete ingestion and, subsequently, death of one of the cells.

but also a new and provocative cell–cell interaction process, which clearly merits further investigation. ■

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COSMOLOGY

An ancient view of acceleration

Michael A. Strauss

The Universe is expanding ever faster — the effect of 'dark energy', most astronomers believe. Surveys of how galaxies were distributed in the past could provide precise clues to what is driving this acceleration.

Gravity holds our expanding Universe together, or so astronomers have long assumed. The mutual attraction of galaxies should counteract the expansion of space that started with the Big Bang, causing the galaxies to slow down. But a decade ago, observations of distant supernovae gave evidence that this simple picture is wrong. That evidence has since been strengthened by a series of other cosmological probes, and the Universe's expansion appears actually to be faster now than it was billions of years ago. Understanding this accelerated expansion is the most pressing problem facing cosmologists today. On page 541 of this issue, Guzzo *et al.*[1] give them a new handle on the acceleration: a comparison of the distribution of galaxies in the Universe now and in the past.

Ideas of what is causing the cosmic acceleration fall into two competing categories. The first is that the Universe is permeated by strange stuff known as dark energy that causes gravitational repulsion. The second is that the equations of general relativity — the theory of gravity formulated by Albert Einstein — are flawed and need to be modified. As best we can understand, the only observational effects of the acceleration are on the history of the Universe's expansion and the rate at which the clustering of matter has increased over time. Thus, we have extraordinarily few observational clues to distinguish between competing models. Astronomers are hungry for additional tests.

Like other techniques used so far, Guzzo and colleagues' approach to understanding the physical nature of the acceleration makes use of a fundamental aspect of astronomical observations: because light's speed is finite, the photons we receive now from a far-off galaxy were emitted at some time in the distant past. That makes it possible to observe the Universe when it was much younger than it is today. By observing galaxies at different distances, we can see how the Universe's properties have evolved as it has expanded.

One of the manifestations of the Universe's expansion is that features such as absorption lines in the spectrum of light emitted by a galaxy are systematically shifted to longer wavelengths — the phenomenon called redshift — by an amount approximately proportional to its distance. Measuring the distance of a galaxy from us is therefore straightforward if one has measured its spectrum.

This situation is somewhat complicated by the fact that, in addition to motion caused by the overall expansion of the Universe, galaxies attract each other gravitationally. In particular, a region of space with more than the average concentration of matter will attract galaxies to it, giving rise to motions that contribute to their redshifts. These motions cause a subtle, but measurable distortion in maps of the galaxy distribution. Determining the amount of this distortion both in the nearby (that is, present-day) and in the distant (early) Universe allows us to learn how the clustering of matter has changed with time (Fig. 1). That yields another clue to the nature of the cosmic acceleration[2].

Guzzo *et al.*[1] measured redshifts of a large sample of distant galaxies using the 8-metre-aperture Melipal telescope in the Chilean Andes (Fig. 2, overleaf). With such a large telescope, they were able to obtain redshifts of almost 6,000 extraordinarily faint galaxies, so distant that we see them as they were when the Universe was only about half its present age, about 7 billion years ago.

The authors measured the distortion in the clustering of these galaxies, and compared it with values from surveys carried out in the nearby Universe. The error bars on the measurements are large, and all that can be shown is broad consistency with currently favoured cosmological models, in which dark energy is responsible for the accelerated expansion. But the authors also show that the next generation of surveys, which will cover 100 times the volume, will have the potential to distinguish between competing models, and provide the explanation for the accelerated expansion of the Universe: dark energy, a change in our understanding of how gravity works, or something even more exotic.

Astronomers are currently gearing up for the next such large redshift surveys. The DEEP2 survey, carried out using one of the two Keck telescopes situated at the summit of Mauna Kea on Hawaii, has observed 40,000 galaxies and has probed roughly the same cosmic epoch[3]. The third

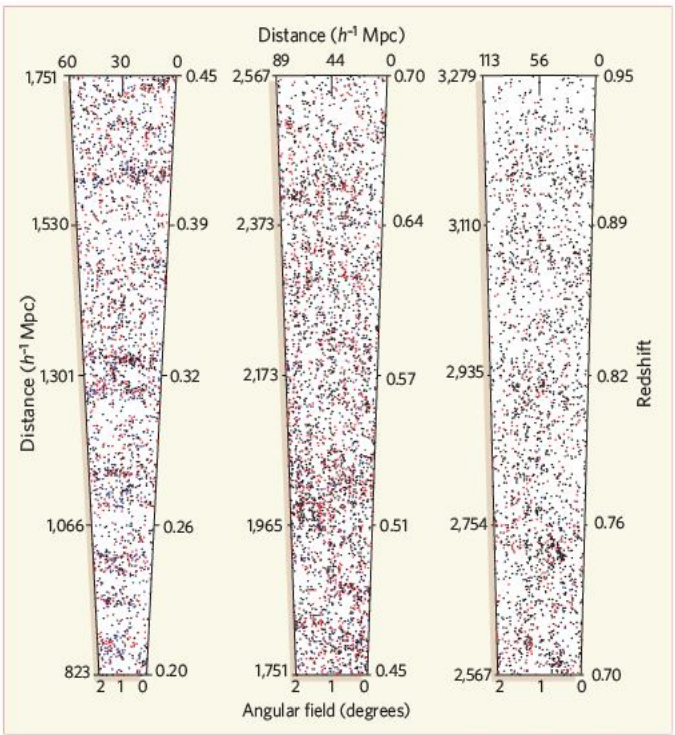

Figure 1 | Clustering over cosmic time. Guzzo and colleagues' survey[1] of galaxy clustering represents a 'pencil beam' covering a region 2° across on the sky, stretching away from us in distance and time (here broken into three contiguous chunks). Each of the 9,126 dots represents a single galaxy. Distances are given in megaparsecs (Mpc; 1 Mpc is about 3.26 million light years) multiplied by h^{-1}. This parameter represents the uncertainty in the Hubble constant, which is a measure of the rate of expansion of the Universe. Distance and time are also measured in terms of redshift z, with $z = 1$ corresponding to a lookback time of roughly 8 billion years. Guzzo *et al.* derive their constraints on cosmological models by examining subtle differences between the clumping of the galaxy distribution in the recent (small z) and ancient (high z) Universe. (Figure from ref. 9.)

C. MARINONI *ET AL.*

ESO

Figure 2 | Very large telescopes. The four telescopes of the European Southern Observatory's Very Large Telescope array adorn the 2,635-metre summit of Cerro Paranal in the Chilean Andes; the Pacific Ocean, at a distance of 12 kilometres, can be seen in the background. The telescopes are named after objects in the sky in the local Mapudungun language: from the left at the back Antu (the Sun), Kueyen (the Moon) and Melipal (the Southern Cross); in the foreground is Yepun (the 'evening star', interpreted as meaning Venus). Guzzo *et al.*[1] used the 8-metre-aperture Melipal telescope for their survey of galaxies so distant that we see them as they were at half the Universe's present age.

phase of the Sloan Digital Sky Survey[4], based in New Mexico, is planned to include 1.5 million galaxies over the next six years at a somewhat more recent epoch than Guzzo and colleagues' survey. Other large surveys are planned or proposed using the Hobby–Eberly Telescope in Texas[5], the Subaru Telescope located next to Keck on Mauna Kea[6], and various space-based missions[7,8]. All are motivated, at least in part, by the desire to study cosmic acceleration. The technique described by Guzzo *et al.*[1] shows that these surveys will be even more powerful than was hoped in constraining the nature of that puzzling phenomenon. ■

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ION CHANNELS

Coughing up flu's proton channels

Christopher Miller

Two research teams have captured snapshots of the influenza virus's membrane-bound hydrogen-ion channel, which is essential for infection and virulence. Their findings agree on the basics, but differ in details.

The paperstorm of research directives blowing out of our biomedical policy offices sometimes drives me to take emotional shelter in a precept of one of my old professors: "Applied research, applied well, soon becomes basic research." The common flu virus illustrates the broad wisdom of this maxim. Influenza and its human vector, playing a cat-and-mouse game for millennia, have come to a rather civilized understanding: you spread me around and I'll spare you to cough another day. Now that the bird-flu media hysterics have died down, we can once again view influenza dispassionately, as a prevalent disease carried by a low-virulence virus with a nasty penchant for — just to keep the game interesting — infrequent but lethal tantrums. This familiar malady has motivated decades of applied (now coyly termed 'translational') research that, instead of a magic bullet, has yielded deep insights across a broad swath of molecular mechanisms in biology, from RNA-based information-processing to weapons of immunological destruction to membrane fusion.

Two papers in this issue further exemplify the link between clinical research into influenza and basic discovery. Stouffer *et al.*[1] (page 596) and Schnell and Chou[2] (page 591) unveil high-resolution structures of M2, an ion-channel protein whose proton (H^+)-conducting activity in the membrane of the influenza virion is necessary for infection. The structures portray our first views of a H^+-specific channel, and they suggest how the virus has outfoxed amantadine, an anti-flu drug effective ten years ago but now well-nigh useless.

An influenza virion engulfed by a lung epithelial cell initially finds itself caged within an intracellular compartment, the endosome. When the virion's membrane fuses with that of the endosome — a process triggered by the acidic endosomal milieu — its RNA genome escapes into the cell to replicate and wreak collateral damage. But for this to work properly, acid must enter the virion through a 'leak' pathway in its membrane — the M2 channel[3,4].

Proton currents mediated by M2 have been described through electrophysiological studies[4]. Earlier work also showed that M2 assembles into a membrane-spanning tetramer[5] to form the channel, which opens and closes at low and neutral pH, respectively. Each of the four protein subunits of the channel is seemingly simple, with only 97 amino-acid residues and a single transmembrane helix.

The M2 structures emerge from complementary high-resolution techniques applied to differently truncated versions of this channel. To solve the structure of the transmembrane peptide of M2, Stouffer and colleagues[1] used X-ray crystallography, and for capturing the image of a longer peptide that includes 15 residues following the transmembrane region, Schnell and Chou[2] used nuclear magnetic resonance (NMR) spectroscopy. Casually viewed, the two structures, which were determined in the presence of amantadine-like inhibitors, agree well. Each is a four-helix, cone-shaped bundle with a polar, proton-friendly pore running along a central axis that is topped by a constriction too narrow for any other type of ion to pass (Fig. 1). Moreover, two functionally 'hot' residues — the gate (Trp 41), which opens when the proton sensor (His 37) experiences low pH — occur at locations that make sense[4]. The NMR structure is apparently a closed state, as the four inward-pointing Trp 41 side chains occlude the pore, whereas the X-ray structure seems to be open, with the helices splaying out on the cytoplasmic side to widen the Trp 41 gate.

Despite this general agreement, however, these papers are going to generate sharp controversy, as is intimated by the gentle whiffs of tendentiousness appearing sporadically

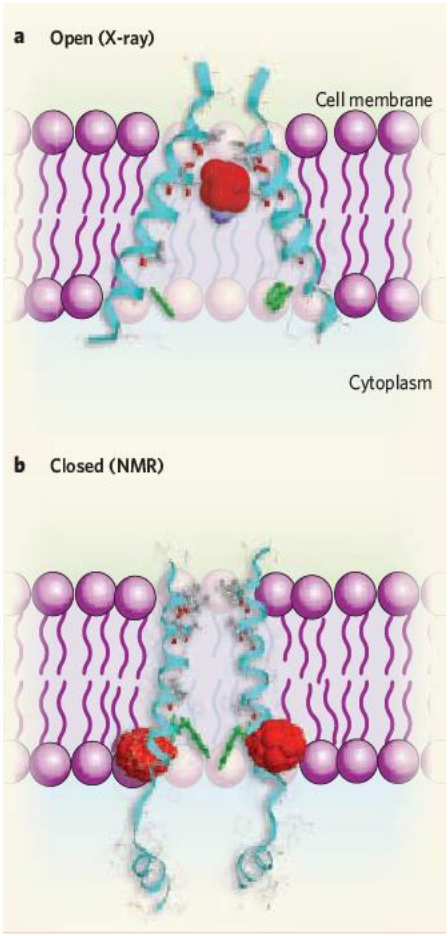

Figure 1 | Depictions of the M2 proton channel. Stouffer *et al.*[1] used X-ray crystallography to determine the structure of the influenza-virus proton channel in the open conformation (**a**), whereas Schnell and Chou[2] solved that same structure in the closed conformation using NMR (**b**). Both structures were determined in the presence of amantadine-like inhibitors (red). For clarity, each structure, imagined in a lipid-bilayer membrane, shows only two of the four M2 subunits that make up the tetrameric channel. Drug binding in the open state blocks the channel, and in the closed conformation it stabilizes the channel. Grey sticks represent positions where natural mutations lead to amantadine resistance. Green sticks show the side chains of the Trp 41 residue that act as the gate to the channel.

in each. The controversy stems not so much from incongruities in structural detail — the channels, after all, are thought to reflect different gating conformations — but from the incompatible amantadine-inhibition mechanisms inferred. The disagreement is not subtle. The X-ray structure shows a single amantadine molecule plugging the open pore. The NMR structure, with no room in the closed pore, surprises us with four drug molecules bound at the channel's lipid-exposed outer surface, one at each helix–helix interface. Each picture suggests its own inhibitory mechanism. Stouffer *et al.* envision the drug physically blocking the H^+ pathway in the open pore. Schnell and Chou, in contrast, propose that the drug binds preferentially to, and thereby stabilizes, the closed state. (I avoid employing the once-precise word 'allosteric', now so carelessly overused as to have become almost meaningless.) Both mechanisms are known to be used by various inhibitors of other ion channels.

Each team cites evidence in favour of its own proposed mechanism. In the now common amantadine-resistant flu strains[6], M2 mutations at any one of six positions in the transmembrane region impair drug inhibition of the channel[4]. In the open structure, four of these residues project side chains into the pore near the amantadine-blocking site, where substitutions could plausibly disrupt drug binding. The closed structure, by contrast, shows three of these side chains projecting sideways to suture the helix–helix interfaces together, far away from the bound amantadine; mutations here could plausibly destabilize the closed state and hence disfavour amantadine binding.

But the correspondence is not perfect, as several of these six side chains project in the wrong direction for each proposed mechanism. I don't consider this a serious weakness, however, as psychoanalysis of protein mutations, even with computational assistance, is an uncertain undertaking. More directly relevant to the issue are several details of amantadine action on full-length M2, but even here neither candidate gets the vote. A stoichiometry of one amantadine molecule per tetramer, inferred indirectly from earlier studies[7,8], naturally fits the X-ray but not the NMR picture. But amantadine action is faster at neutral pH, where the channel is mostly closed, than at low pH, where the open state is favoured[7], in accord with closed-state stabilization but not with open-pore block.

These and other ambiguities abound, not least of which is the embarrassment that neither truncated construct used for the structures has been certified for proper H^+-channel function. It had been convincingly shown[9] that the minimal transmembrane peptide forms amantadine-binding tetramers in detergent solution. But the literature is littered with disquieting variability in this peptide's ion-transport function in membranes; single-channel turnover rates, for instance, range from 10 to 10^7 ions per second (in one case[10], this millionfold discrepancy appearing in the same figure). So although the new structures[1,2] give us consistent glimpses of this fascinating proton channel's overall architecture, they clash in their mechanistic inferences. As for representations of the M2 channel in a biological membrane, the discrepant views cry out for resolution, which will require further structural work combined with its essential companion, close functional scrutiny. ■

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DEVICE PHYSICS

Nanowires' display of potential

Hagen Klauk

The future of the video display is both flexible and transparent. Finding a material for the attendant electronics that is small-scale, bendy and see-through is a tall order — but a promising candidate is emerging.

Semiconductor nanowires are diminutive semiconductor crystals, generally a few micrometres long and just 20–80 nm in diameter, and could be the bedrock of next-generation integrated circuits: ultra-scaled microprocessors, terabit memory chips and the like. The hope is to exploit the nanowires' tiny dimensions to construct such devices at reasonable cost by cramming as many as 200 billion nanowire transistors onto each square centimetre of silicon substrate — which will also demand a departure from the traditional planar transistor layout[1].

That remains a long-term goal. In the meantime, the signs are that nanowire transistors are already carving out a niche for themselves in applications requiring much smaller integration densities and lower switching speeds. To take one example, Ju *et al.*[2] show in *Nano Letters* that the unique properties of nanowire transistors make them an excellent choice for active-matrix displays made from organic light-emitting diodes (OLEDs).

In such displays, each pixel must contain several transistors along with the actual OLED. The transistors are there to ensure that all pixels emit the desired colour at the desired brightness, even when the image changes rapidly during full-motion video replay. The number of transistors per unit area in a display is

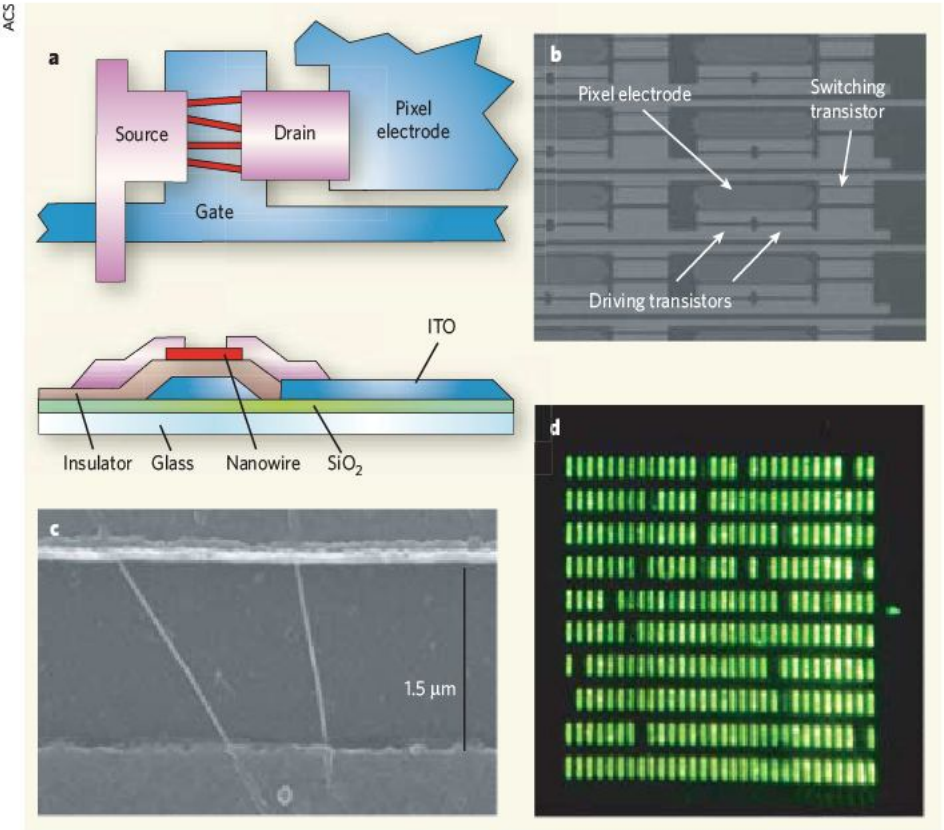

Figure 1 | Small, flexible and see-through. **a**, At the heart of Ju and colleagues' transistor device[2] for the pixels of an organic light-emitting-diode (OLED) active-matrix display are indium oxide nanowires that connect the source and drain of the transistor, made of the transparent conductor indium tin oxide (ITO). The nanowires are separated from the transistor's gate electrode by a thin molecular insulator, meaning that small voltages are sufficient to control the transistor. **b**, The structure, sitting atop a silicon dioxide (SiO_2) layer on a glass substrate, minimizes the area lost to the OLEDs of the display: in this top-down view of several 54 μm × 176 μm pixels, the rounded lozenges are the OLED electrodes that take up about 46% of the available area (the aperture ratio of the display). **c**, A scanning-electron microscope image of the nanowires (slanting lines) inside the device. **d**, A 2 mm × 2 mm display when operated at a transistor-operation voltage of 3 V and an OLED drive voltage of 5 V. (Figure adapted from ref. 2.)

comparatively small (around 10,000 per square centimetre), and so the area taken up by each transistor is less important than in a memory or microprocessor chip.

The practical implication is that transistors in an OLED display can use semiconductors, such as amorphous silicon[3,4] or conjugated oligomers[5], in which the mobility of charge carriers is relatively low. Small mobilities mean that the transistors must be large (that is, wide) to transport enough carriers and drive the necessary currents (about 10^{-5} amps per pixel). The great advantage of these semiconductor materials is that they can be processed at temperatures low enough to permit the use of polymeric substrates — substrates that can be both flexible and transparent.

In reality, however, the transistors in a display cannot be as large as you please: the more space taken up by the transistors in a pixel, the less area is actually available for emitting light. An important goal of display development is to increase the aperture ratio — the fraction of the total pixel area available for light emission. With amorphous silicon or organic transistors and a pixel size of 300–500 μm, aperture ratios of 40–50% are possible[3–5].

To boost the aperture ratio, the first mass-produced active-matrix OLED displays[6] use transistors based on polycrystalline silicon. The significantly larger charge-carrier mobilities in this semiconductor, compared with those in amorphous silicon or organic semiconductors, permit narrower transistors and a larger aperture ratio. But polycrystalline silicon transistors also require higher processing temperatures, making it difficult to use polycrystalline silicon with polymeric substrates.

And so semiconducting nanowires come into play. Because they consist of just a single crystal, carrier mobilities are high: Ju *et al.* report[2] a value of 250 $cm^2 V^{-1} s^{-1}$ for their nanowires made of indium oxide, In_2O_3. A small assembly of properly aligned nanowires thus delivers the same drive current as a polycrystalline silicon transistor.

Ju and colleagues prepared their nanowires (Fig. 1) by pulsed laser ablation, which involves vaporizing material from a bulk solid target using a laser, and transferred them to the display substrate from a liquid suspension. Once on the display substrate, the authors connected the nanowires into transistors and pixel circuits using standard photolithography methods. Excess nanowires were shaken off in a bath using ultrasound.

By employing an optically transparent conductor for the source and drain contacts (the entry and exit points for current flowing through the transistor) and for the gate electrodes (which control the magnitude of that current), transistors were obtained that will be useful for see-through displays — on car windscreens, for example. The gate electrode must be isolated from the nanowires by an insulating layer, and for this the authors used a very thin molecular dielectric with very few defects and a large capacitance (Fig. 1a). This large capacitance means that the gate voltage required to induce a sufficient number of carriers in the nanowire is, at about 4 volts, relatively small, minimizing the power consumption of the display.

Because the nanowires are not produced on the display, but are transferred to the substrate after preparation, the thermal budget for preparing the semiconductor is decoupled from the thermal budget for transistor fabrication. Thus, even if high energies or temperatures are required to prepare nanowires with sufficient carrier mobility, the display can be made on low-temperature polymeric substrates. The maximum temperature during the authors' transistor fabrication process was about 100 °C (which was the temperature required for the photolithography involved). Transistors based on high-mobility polycrystalline silicon films, by contrast, require either a furnace anneal at about 500 °C or time-consuming laser crystallization. What is more, as Ju *et al.*[2] show, nanowires work very well with organic gate dielectrics that can be prepared at room temperature. Amorphous and polycrystalline silicon, on the other hand, normally require inorganic dielectrics deposited at temperatures of about 200 °C.

Considering their relatively small pixel size (54 μm × 176 μm) and the lack of a specific nanowire placement strategy, which means that only a small fraction of the transistor area is available for electron transport, the displays reported by the authors have a very respectable aperture ratio of 46% (Fig. 1b). So far, the displays are monochrome rather than full-colour, and the response is for static pixel operation rather than video-rate matrix addressing. Even so, the demonstration of an active-matrix OLED display with nanowire transistors has substantial implications: all that is required now is an efficient nanowire placement process (and perhaps a few improvements in OLED efficiency and lifetime), and high-quality flexible displays might finally become a reality. ■

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ARTICLES

Predicting expression patterns from regulatory sequence in *Drosophila* segmentation

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The establishment of complex expression patterns at precise times and locations is key to metazoan development, yet a mechanistic understanding of the underlying transcription control networks is still missing. Here we describe a novel thermodynamic model that computes expression patterns as a function of *cis*-regulatory sequence and of the binding-site preferences and expression of participating transcription factors. We apply this model to the segmentation gene network of *Drosophila melanogaster* and find that it predicts expression patterns of *cis*-regulatory modules with remarkable accuracy, demonstrating that positional information is encoded in the regulatory sequence and input factor distribution. Our analysis reveals that both strong and weaker binding sites contribute, leading to high occupancy of the module DNA, and conferring robustness against mutation; short-range homotypic clustering of weaker sites facilitates cooperative binding, which is necessary to sharpen the patterns. Our computational framework is generally applicable to most protein–DNA interaction systems.

Precise spatio-temporal control of gene expression lies at the heart of metazoan development. The necessary instructions are encoded in *cis*-regulatory elements, or modules, which typically contain multiple binding sites for multiple transcription factors[1]. When bound, transcription factors promote or inhibit expression of the neighbouring gene, with the net expression outcome determined by how all factor effects integrate. The binding of factors depends on their affinity to the binding sites, but also on their expression levels; because these vary spatially and temporally, the constellation of bound factors on the module sequence and the resulting expression level will vary accordingly. Thus, understanding the rules by which modules 'compute' expression from the input factor expression is key to understanding transcriptional processes in general and pattern formation in particular.

Genetic, molecular and biochemical studies, more recently complemented by ChIP-chip[2] and by computational approaches exploiting site clustering[3–5], conservation[6] or co-regulation detected by DNA microarrays[7], have collectively identified many of the genes, modules and binding sites involved in key developmental processes. To unravel the logic by which these components interact, various types of logical[8], probabilistic[7,9], thermodynamic[10–12], and reaction–diffusion models[13–15] have been constructed, providing interesting insights. However, these methods do not explicitly model transcription factor binding to regulatory sequence, or do so on a limited scale[10,12,16]. Thus, a quantitative mechanistic description of the transcriptional control events that lie at the core of developmental pattern formation is still missing.

Here we present a new computational framework that models the entire process of transcriptional regulation, from the expression of the input factors to their binding to *cis*-regulatory sequence and the module expression patterns resulting from these binding events. The model is based on physical properties and takes into account binding competition between factors, cooperative binding interactions, and contributions from weak binding sites. We apply our framework to the well-characterized segmentation gene network of the early *Drosophila* embryo, which consists of a four-tiered hierarchy of maternal and zygotic factors that define the antero-posterior body axis in a stepwise refinement of expression patterns[17–20]. The maternal factors form gradients spanning the entire antero-posterior axis; they are translated into broad, non-periodic domains of zygotic gap gene expression and subsequently into periodic patterns of seven 'pair rule' and finally fourteen segmental stripes that prefigure the fourteen segments of the larva. Regulation within this network is highly combinatorial and, in the top tiers, almost entirely transcriptional.

Thermodynamic model of transcription control

Our model takes as input expression levels and DNA-binding specificities for a set of transcription factors, and predicts the expression level that any arbitrary DNA sequence will give rise to when receiving input from these factors (Fig. 1). The model has two main components: one that computes the occupancy distribution of factors on a given target DNA sequence, and another that translates this occupancy distribution into a level of expression. To account for differing input factor concentrations, these computations are performed separately for every position along the spatio-temporal axis of interest, here the antero-posterior axis.

In the first model component, we consider all possible configurations of factor molecules on the sequence; by not allowing overlap between two molecules in any one configuration, we model the competition between factors that results from their steric hindrance constraints (Fig. 1, and Supplementary Fig. 1). The probability of a configuration is computed from the local concentration of the participating factors and the strength of the binding sites they occupy in the configuration, as measured by the position specific scoring matrix (PSSM) score[21]. We do not use predetermined thresholds for defining factor-binding sites, allowing both weak and strong factor binding to contribute, and we model self-cooperativity between two factor molecules bound to neighbouring sites, assuming that this effect decays with the distance between the sites. The second model

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component translates each configuration into its resulting expression level. We assume that each bound factor molecule contributes independently to the expression outcome, with activator molecules contributing positively and repressor molecules contributing negatively. We use the logistic function to translate these contributions into expression because it has the desired saturation properties whereby maximal or minimal transcription is achieved beyond a certain number of bound activator and repressor molecules, respectively. The final expression outcome of a sequence is then the sum of the expression contributions of each configuration, weighted by their probability (see Supplementary Information).

The model has three free parameters for each transcription factor, representing values that are typically unknown: (1) the absolute concentration of the factor *in vivo*; (2) the transcription rate resulting from its interactions with the basal machinery; and (3) the strength of binding cooperativity for the factor. In addition, we parameterize the PSSMs representing the factors' binding preferences because they are typically based on a limited number of footprinted binding sites, but we constrain PSSM learning to maintain the measured consensus (see Methods). We devised a learning algorithm that fits the model parameters to minimize the error between the measured and model-predicted expression for a set of input modules. This model-fitting task is complex because it requires traversing the uncomputably large number of possible factor configurations on the sequence, and calculating the expression contribution of each such configuration. To approximate this computation, we devised a sampling-based algorithm, guaranteed to converge to the correct computation as the number of samples increases.

Modelling pattern formation in segmentation

To apply our model to the segmentation network, we used as input the spatial expression patterns[22] for eight key transcription factors, that is, Bicoid (BCD), Hunchback (HB), Caudal (CAD), Kruppel (KR), Giant (GT), Torso-response element (TorRE), Knirps (KNI), Tailless (TLL), and their binding-site preferences[5,23]. We asked the model to predict the spatial expression of 44 gap and pair-rule gene modules with known patterns, collated from literature and from our own work[5]. We modelled the input–output relationship for one developmental time point, using a time at which both the input factor patterns and the output module expression patterns are mature (mid-blastoderm; approximately 20 min into cell cycle 14).

The expression patterns predicted by a model trained on these data exhibit good or fair agreement with the measured patterns for most modules (Fig. 2a). The parameters behave in a biologically plausible fashion: fitted values typically differ by less than an order of magnitude between the different factors, and the trained PSSMs show only small changes from their original settings (Fig. 3a, and Supplementary Fig. 2). The expression of gap gene modules is generally predicted very well, suggesting that our model has adequately captured their input and rules. In contrast, prediction of pair-rule gene modules is more mixed, with failures resulting from missing activation (modules receiving little maternal activator input), or occasionally from ectopic expression that is due to missing repression, mostly in the head region of the embryo (Supplementary Fig. 3). Because our model includes only self-cooperative interaction, we also fail to predict the module generating *even-skipped* (*eve*) stripe 2 (*eve_2*), which is known to require positive synergy between BCD and HB[24]. Overall, the failures of our model are as instructive as its successes—they suggest that some input factors and some higher interaction rules are not captured, but also that the model does not artificially compensate for these missing features.

Model validation

A critical test for our model is whether it can predict the expression patterns of modules that were not used as input when fitting the parameters. We used two such sets of held-out modules: 11 recently published anterior modules[4], and 15 segmentation modules from the related species *D. pseudoobscura*, which we identified and tested in a separate study (S. Sinha *et al.*, manuscript in preparation). The expression of *D. pseudoobscura* modules was measured using transgenic reporter constructs in *D. melanogaster*, such that all observed effects were attributable to module sequence. While producing only mild to moderate changes in expression, the *D. pseudoobscura* modules show substantial sequence turnover compared to their *D. melanogaster* orthologues (average sequence identity 49%; Supplementary Fig. 4) and thus represent a profound *cis* perturbation. For both test sets, our model, using the parameters trained on the original 44 modules, predicts expression with mostly good or fair

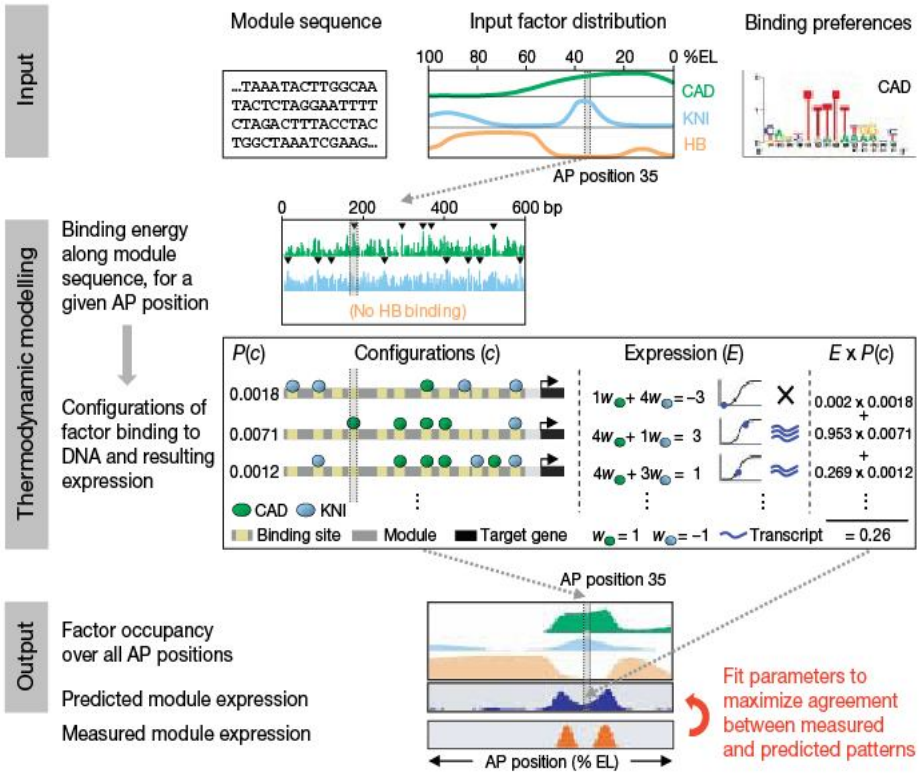

Figure 1 | Overview of the thermodynamic model and approach. Flow diagram showing input, output and the main steps of the computational framework, for a simplified version of the *eve_4_6* module with three transcription factor inputs. The computation is shown for one particular position along the antero-posterior (AP) axis, measured as percentage of egg length (%EL). At each antero-posterior position, the factor concentrations (top panels) define a binding energy landscape for all factors across the module sequence, which is then translated into a factor occupancy distribution (middle panel). Each factor configuration, *c*, results in a particular expression level, *E*, represented as a fraction of the maximal achievable transcription rate and calculated from the number of transcription factors bound in the configuration and the factor-specific expression contribution parameters w_{tf}, using the logistic function. The overall resulting expression outcome at each position (bottom panel) is then computed as the sum of the expression contribution of each configuration, weighted by their probability *P(c)*. For a detailed description see main text and Supplementary Information.

accuracy; the success rate is similar to that obtained with the training data (Fig. 2b, c). Notably, with few exceptions, modules that are predicted well in *D. melanogaster* are also predicted well in *D. pseudoobscura*, highlighting the model's intrinsic consistency.

As a further test, we conducted a standard tenfold cross validation assay, using an automated objective performance measure that scores the expression predictions at each antero-posterior position as 'on' or 'off', depending on whether they are above a certain threshold, and iterates over all possible thresholds. The resulting sensitivity/specificity plots reveal that our model performs much better than random expectation or models using various types of randomized weight matrices; similar results are obtained when applying this automated performance assessment to the above two sets of held-out modules (Supplementary Fig. 5). Taken together, our validation tests provide strong evidence that the successful predictions are not the result of overfitting the input data, and thus suggest that our model indeed captures core principles governing pattern formation in the segmentation network.

Weak sites and cooperativity

Our model predicts a high occupancy of factors on the modules: on average, 14–27% of the module DNA is occupied by factor molecules, with some variation along the antero-posterior axis (Fig. 4a). Translated into binding events, this suggests an average of 10–60 bound molecules per module at each antero-posterior position, depending on module length. This high occupancy is consistent with the results of footprinting experiments and genome-wide chromatin immunoprecipitation of segmentation and other factors[25–27]. Much of the occupancy is attributable to factor binding to moderate or weak binding sites. Although modules are enriched in stronger sites, such sites collectively account for only about half of the total factor occupancy; the other half comes from weaker sites that occur no more frequently than is expected by chance and whose PSSM scores place them at the low end of the range defined by footprinted sites (Fig. 3b). Interestingly, models that exclude weaker binding sites have lower predictive power (Supplementary Fig. 5b), demonstrating their importance for pattern formation. The use of many contributing sites may help to reduce gene expression noise by increasing the frequency of activation steps[28], and confer robustness to the module expression pattern against point mutations and small deletions: by *in silico* mutational analysis, we find that point and small deletion mutations are tolerated in 8 to 15% of total module length, with little effect on the resulting expression patterns (Supplementary Fig. 6).

Low affinity binding sites will frequently occur by chance within the length of a typical module, but specificity may be increased by clustering. Indeed, we find that 5 of the 8 transcription factors participating in the system show significant short-range homotypic clustering of binding sites within the modules, typically within 200 bp (Fig. 3c); the only exceptions are factors for which the available binding site information is either relatively unspecific or very sparse (see Methods). We observe no systematic heterotypic clustering between binding sites of different factors. An important feature of homotypic site clustering is that it facilitates cooperative binding, which plays an important role in transcriptional switches[29] and leads to a sharpening of expression patterns in BCD-dependent modules[30,31]. Notably, when cooperativity effects are disregarded, our model predicts expression patterns with a very gradual decay along the antero-posterior axis, in contrast to the sharp boundaries of the measured expression profiles and indeed to the much sharper patterns predicted by the full model (Fig. 4b, and Supplementary Figs 7, 8); this suggests the pervasive use of cooperativity in segmentation. We do not know how cooperativity is achieved mechanistically—by homotypic protein–protein interactions, transcriptional synergy, or perhaps competition with nucleosomes[32,33]—but the similar narrow range within which the clustering occurs for most factors suggests a general common mechanism.

Design principles of segmentation

Earlier work on individual modules[34–37] had shown that their expression patterns are generated by combinatorial action of input factors, with maternal factors acting as activators and gap factors mostly as

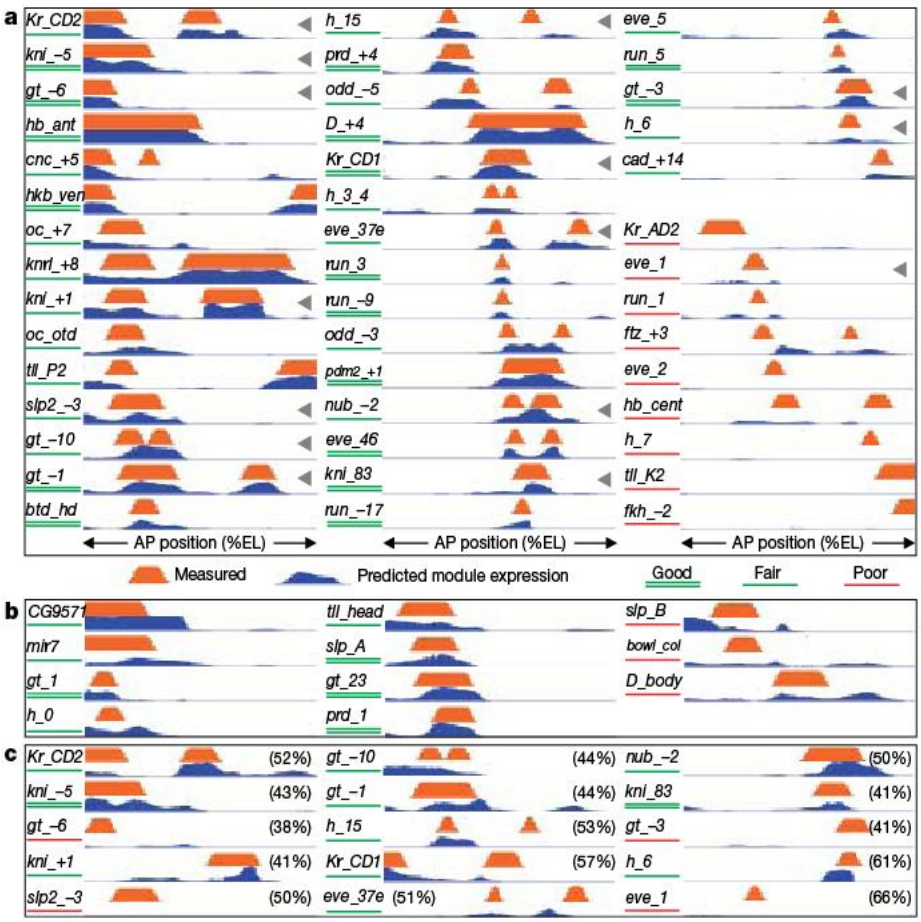

Figure 2 | Predicted expression patterns and model validation. **a–c**, Comparison between measured module expression patterns (red) and those predicted by the model (blue) for all 44 modules used to fit the parameters (**a**), as well as for modules not used for parameter fitting (**b**, **c**): **b**, 11 recently identified anterior modules[4] (note that *gt_23*, *gt_1*, *prd_1* and *D_body* represent shorter delineations of our modules *gt_−10*, *gt_−6*, *prd_+4* and *D_+4*, respectively); **c**, Fifteen modules from *D. pseudoobscura* (S. Sinha *et al.*, manuscript in preparation). Sequence identity as determined by pairwise sequence alignment is indicated in parentheses; the orthologous *D. melanogaster* modules are marked by grey triangles in **a**. Modules were subjectively classified into three categories (good, fair, poor) on the basis of the quality of the match between measured and predicted pattern and the amount of spurious expression.

repressors. The results of our modelling support these general notions, but argue against other previously suggested design principles. Our model classifies the maternal factors BCD, CAD and TorRE as activators, and the zygotic gap factors (HB, GT, KR, KNI, TLL) as repressors (Fig. 3a), suggesting that context-dependent function, which had been proposed for some gap factors[24,35,38–40], is not necessary to account for most expression patterns. The maternal activators show higher total occupancy in modules that are expressed and lower occupancy in modules that are not expressed in the same region as they are, indicating that their prevalent mode of action is indeed activation; the zygotic factors show the converse behaviour (Fig. 3d). Interestingly, both activators and repressors show significant binding in 'inappropriate' modules, albeit at lower levels, suggesting that module design is not entirely parsimonious.

When we examine how the expression patterns of individual modules are generated, we find that all modules are highly combinatorial in design and generally contain one or two types of activating input and multiple repressive inputs, with preference for co-extensive activator(s) and against co-extensive repressors: modules typically receive activation from the activator most appropriate for their region, with some choice in the middle (BCD/CAD) and at the termini (BCD/TorRE or CAD/TorRE) of the embryo; the choice of activator(s) entails the choice of appropriate repressors. An illustrative example for these design features is the differential factor occupancy in the modules generating the two main expression domains of the gap gene *gt* (Fig. 5, and Supplementary Figs 9, 10). Modules generally disfavour but do not exclude sites for co-extensively expressed repressors; sites for the cognate factor, however, are very rare: of the 11 modules driving the expression of the primary gap gene domains, only 3 have significant input by the factor itself (*gt_−6*, *Kr_CD2*, *tll_K2*) (Supplementary Fig. 10). This argues against a significant role for direct auto-regulation of gap factors in the patterning, which had figured prominently in several theoretical models[15,41].

Interestingly, we find no (positive) correlation between the strength of BCD input and the posterior border of target module expression (Fig. 4c). This finding suggests that the number/quality of BCD-binding sites in the target modules is not the sole determinant of position in the anterior portion of the embryo as envisaged by the gradient–affinity model of BCD action[42,43]; rather, module expression boundaries seem to be determined as much by repressive gap gene input as by attenuation of maternal activation[4].

We find little overlap between the binding preferences of the different input factors, and as noted above, no heterotypic clustering. This suggests that the different factors bind to the DNA largely independently and that sequence-specific competition or occlusion, which had been proposed as a mechanism of repressor action[35,44,45], does not play a major role. The one exception is the strong overlap in binding preferences between HB and CAD (Fig. 4d, and Supplementary Fig. 11), which is in fact exploited in the design of many posterior modules: because the two factors have opposing

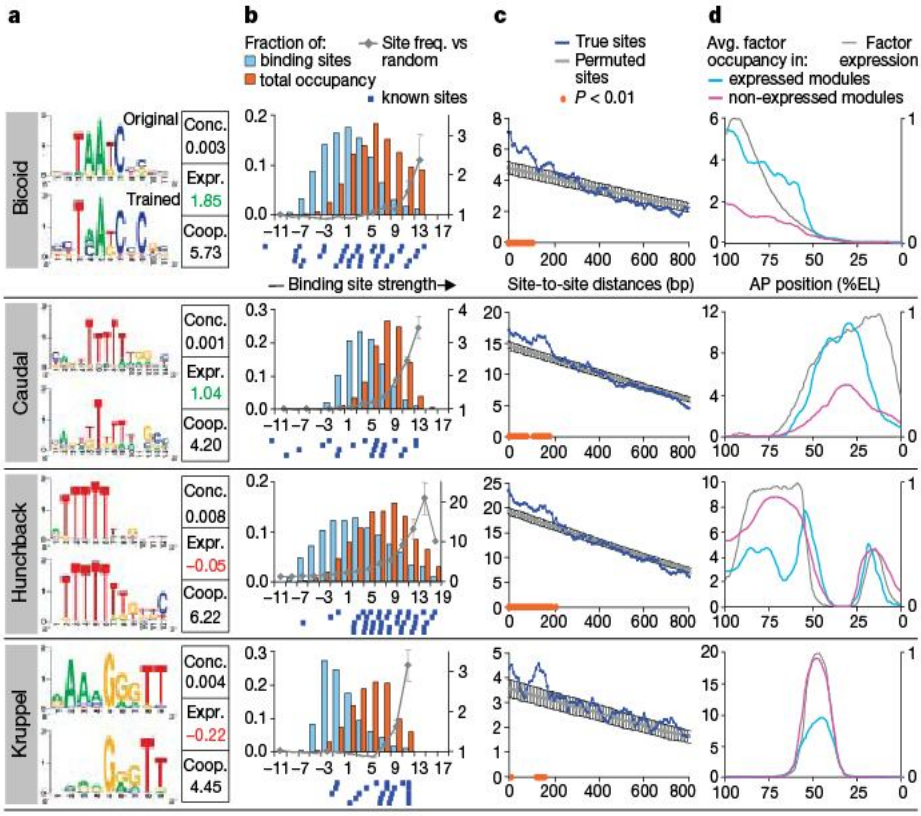

Figure 3 | Participating transcription factors and their behaviour. **a**, PSSMs representing binding preferences before (upper panels) and after (lower panels) training and other parameters (absolute factor concentration, expression contribution and self-cooperativity) as fitted by our model, for four key transcription factors regulating segmentation (see Supplementary Fig. 2 for all eight factors). **b**, Binding-site strength and contribution to occupancy. For each factor, histograms show as a function of site strength: the number of binding sites (expressed as fraction of all sites, light blue columns, left scale), and the fraction of the total factor occupancy contributed by sites of this strength (orange columns, left scale). Binding-site strength is defined as the log-ratio between the PSSM and background model score of the site[21], using a uniform background. Over-/under-representation of sites in modules (grey line, right scale) is calculated as the ratio of the number of sites of a given strength in the actual module sequence versus the number of such sites in randomly permuted module sequence (shown is mean ± s.d. computed from 100 permutations). The strength of experimentally footprinted sites is represented by dark blue squares below the histogram. **c**, Short-range homotypic clustering of binding sites. Shown is the number of pairwise distances between same-factor sites that are within a range of k to $k + 50$ bp, plotted for values of k from 0 to 800 (y axis) and expressed as average per module (blue line), compared to results of 1,000 permutation tests in which the predicted sites are randomly placed within each module (grey line, mean ± s.d.). **d**, For each factor, shown is the measured endogenous expression (black), compared with the average predicted total factor occupancy in modules expressed at a given antero-posterior position (blue), and with average predicted total occupancy in modules not expressed at that position (pink); note differing behaviour of activators and repressors.

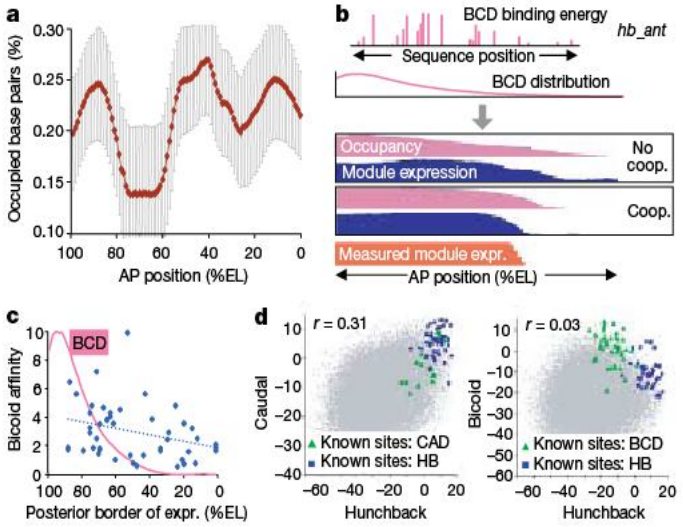

Figure 4 | Cooperative DNA binding and binding site overlap. **a**, Fraction of module sequence (mean ± s.d.) that is occupied by factors at each antero-posterior position, averaged across all modules. **b**, Effects of modelling self-cooperativity for the *hb_ant* module: with cooperative binding permitted, graded BCD input is translated into a much sharper pattern of BCD occupancy and predicted module expression. **c**, Strength of BCD input shows no (positive) correlation with the posterior boundary of module expression. For each module, the position of its measured posterior expression boundary (*x* axis) is plotted against the maximum predicted total occupancy of BCD along any antero-posterior position ('Bicoid affinity', *y* axis); BCD protein distribution is shown as a purple curve. **d**, Scatterplots visualizing the correlation between the binding-site strengths (presented as the log-ratio between the PSSM and background model score) with which two factors bind to each position in the concatenated sequence of all modules. HB and CAD show significant overlap in their binding preferences, whereas HB and BCD do not; see also Supplementary Fig. 11. The known binding sites for the two factors, from which the PSSMs were derived, are highlighted.

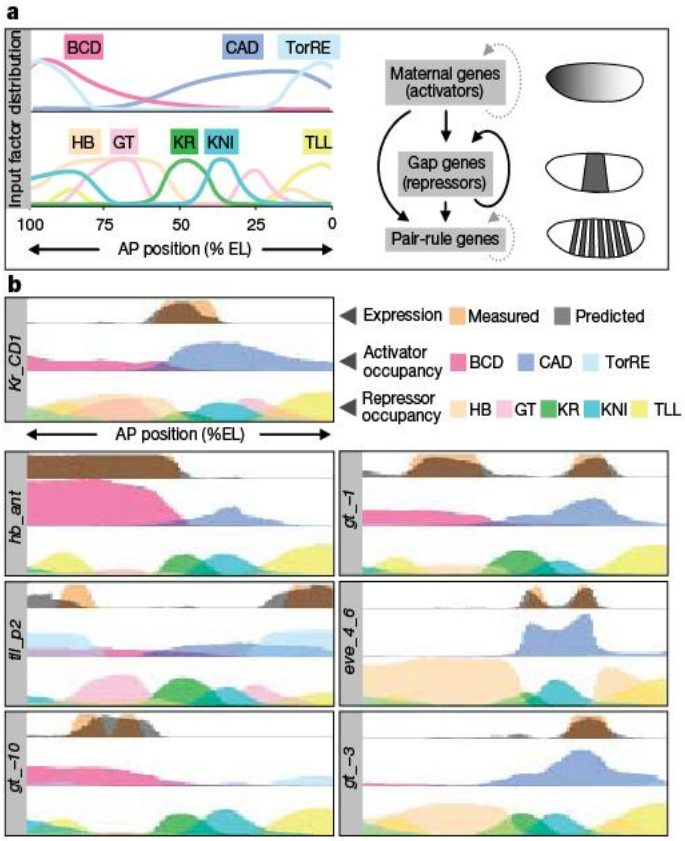

Figure 5 | Regulatory input and expression of segmentation modules. **a**, Distribution of the eight participating transcription factors along the antero-posterior axis, with maternal factors (activators) and gap factors (repressors) plotted separately, and schematic depiction of the global architecture of the segmentation network. **b**, Graphs show the occupancies of the participating transcription factors at every position along the antero-posterior axis for selected modules, thus indicating which factors control expression at a particular position. Occupancy curves are colour-coded by factor; the curves for activators (middle) and repressors (bottom) are superimposed and plotted separately; the resulting predicted expression level (grey) is shown on top, superimposed on measured expression level (orange); see also Supplementary Fig. 10.

expression patterns and functions, a broad abdominal pattern can be generated through differential occupation of overlapping CAD/HB sites along the antero-posterior axis by one or the other factor, with CAD causing activation in the posterior portion of the embryo and HB causing repression in the anterior and near the posterior terminal. Additional repressive input then further narrows module expression boundaries, as seen, for example, in *gt_−3* (Fig. 5, and Supplementary Fig. 10).

The central biological task of the segmentation gene network is to subdivide the embryo along the antero-posterior axis by translating broad maternal gradients into successively narrower and sharper patterns. Our analysis suggests that this is accomplished by parallel combinatorial input of multiple factors and self-cooperative factor interaction. It is intriguing that these crucial design features are embedded locally within the *cis*-regulatory sequence rather than in the *trans*-factor network and the basal machinery, which presumably increases the reliability of the readout and the ability of the system to evolve.

Conclusions

We have presented a quantitative model for transcription control in pattern formation that integrates sequence and expression information and seeks to capture the mechanistic core of the process. Input factors bind DNA at thermodynamic equilibrium, dependent only on their concentration and on the arrangement and quality of their sites within the modules, but without introducing thresholds or other filters. By applying the model to the segmentation network of *Drosophila*, we demonstrate that these principles, in conjunction with uniform and biologically plausible parameters for the unknown aspects of the molecular interactions, are sufficient to produce the patterns of most experimentally validated modules with substantial accuracy, even across species. A notable feature of our approach is that the network structure between factors and their target genes is not pre-defined; rather, we assume a fully connected network in which all possible factor–module interactions are considered and the network structure is an emergent property of the molecular *cis*-regulatory interactions, changing dynamically with the variation in local factor concentrations. Our framework is generally applicable and likely to prove useful for many other protein–DNA interaction systems. Several important issues need to be addressed to improve further the predictive accuracy of our model, such as integrating the temporal evolution of expression patterns, modelling heterotypic synergy (BCD/HB) or other non-additive factor interactions (for example, repressor quenching), identifying missing input factors, and incorporating competition with nucleosomes. The model will also greatly benefit from additional experimentation to constrain parameter values, such as measuring factor cooperativity and improving the PSSMs.

METHODS SUMMARY

Spatial expression patterns and measured binding preferences for eight transcription factors in the segmentation network were obtained from published sources[5,22]; for selected factors, the functionality of the consensus sites was confirmed by insertion into a synthetic enhancer[46]. Expression patterns for segmentation gene modules were collected from published sources[4,5] or our own work and measured as described[5]. The full mathematical details and fitting procedures of our model are described in the Supplementary Information. The significance of local clustering of binding sites was assessed by calculating

the number of all pairwise distances between sites for the factor(s) considered that fall within moving windows of 50 bp, and comparing this to the results obtained when the same number of binding sites are randomly placed within each module. The occupancy of a factor at a base pair and antero-posterior position is defined as the sum of the probabilities of all configurations in which the base is occupied by the factor; the total occupancy contribution of a binding site is then the sum of its occupancy across all antero-posterior positions, and the fractional occupancy contribution of a binding site is equal to its total occupancy divided by the total occupancy of all binding sites for the factor. For input data and results, see our website http://genie.weizmann.ac.il/pubs/segnet08.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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LETTERS

A test of the nature of cosmic acceleration using galaxy redshift distortions

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Observations of distant supernovae indicate that the Universe is now in a phase of accelerated expansion[1,2] the physical cause of which is a mystery[3]. Formally, this requires the inclusion of a term acting as a negative pressure in the equations of cosmic expansion, accounting for about 75 per cent of the total energy density in the Universe. The simplest option for this 'dark energy' corresponds to a 'cosmological constant', perhaps related to the quantum vacuum energy. Physically viable alternatives invoke either the presence of a scalar field with an evolving equation of state, or extensions of general relativity involving higher-order curvature terms or extra dimensions[4–8]. Although they produce similar expansion rates, different models predict measurable differences in the growth rate of large-scale structure with cosmic time[9]. A fingerprint of this growth is provided by coherent galaxy motions, which introduce a radial anisotropy in the clustering pattern reconstructed by galaxy redshift surveys[10]. Here we report a measurement of this effect at a redshift of 0.8. Using a new survey of more than 10,000 faint galaxies[11,12], we measure the anisotropy parameter $\beta = 0.70 \pm 0.26$, which corresponds to a growth rate of structure at that time of $f = 0.91 \pm 0.36$. This is consistent with the standard cosmological-constant model with low matter density and flat geometry, although the error bars are still too large to distinguish among alternative origins for the accelerated expansion. The correct origin could be determined with a further factor-of-ten increase in the sampled volume at similar redshift.

A relevant consequence of the presence of a dominant form of dark energy in the Universe, in addition to its primary effect on the expansion rate, is to modify the gravitational assembly of matter from which the observed large-scale structure originated. In linear perturbation theory, it is possible to describe the growth of a generic small-amplitude density fluctuation through a second-order differential equation. This equation depends on the expansion rate $H(z)$, but also on the theory of gravity. From its solutions, we can define a linear growth rate f that measures how rapidly structure is being assembled in the Universe as a function of cosmic time, or, equivalently, of the redshift. The redshift $z = \lambda_{\rm meas}/\lambda_{\rm emis} - 1$ of the radiation emitted by a distant object is a measure of the time of emission through its dependence on the cosmic scale factor $a(t)$, which is $1 + z = 1/a(t_{\rm emis})$. $f(z)$ essentially depends on the value of the mass density parameter at the given epoch, $\Omega_{\rm m}(z)$, which is defined as the ratio of the matter density $\langle\rho_{\rm m}(z)\rangle$ to the 'critical' density required to halt the expansion $\rho_{\rm c} = 3H(z)^2/8\pi G$, where G is Newton's constant of gravity. For the flat cosmological-constant model (in which the total density in matter and dark energy is $\Omega_{\rm m} + \Omega = 1$) the dependence[9] is $f(z) \approx [\Omega_{\rm m}(z)]^{0.55}$. However, this is not valid if the observed acceleration originates from a modification of the equations of the general theory of relativity; for example, in the Dvali–Gabadadze–Porrati (DGP) braneworld theory, an extra-dimensional modification of gravity[13], $f(z) \approx [\Omega_m(z)]^{0.68}$. In general, a fitting form $f(z) \cong [\Omega_{\rm m}(z)]^{\gamma}$ has been shown to be an accurate description for a wide range of models[9,14] (for which $\Omega_{\rm m}(z)$ itself, not only γ, depends on the model). Thus, models with the same expansion history $H(z)$ but a different gravity theory will have a different growth rate evolution $f(z)$ and index γ (refs 9, 15). A discrepancy between the measured value of the growth rate and that computed independently (assuming the general theory of relativity applies) from the $H(z)$ yielded by type Ia supernovae would point to modifications of gravity[6–8], rather than to exotic new ingredients in the physical content of the Universe[4,5].

A few observational techniques have been suggested to measure $f(z)$ at different redshifts[9,16]. Redshift-space distortions, that is, the imprint of large-scale peculiar velocities on observed galaxy maps, have not yet been considered in this context. Gravity-driven coherent motions are in fact a direct consequence of the growth of structure. The anisotropy they induce in the observed galaxy clustering when redshifts are used as a measure of galaxy distances can be quantified by means of the redshift-space two-point correlation function

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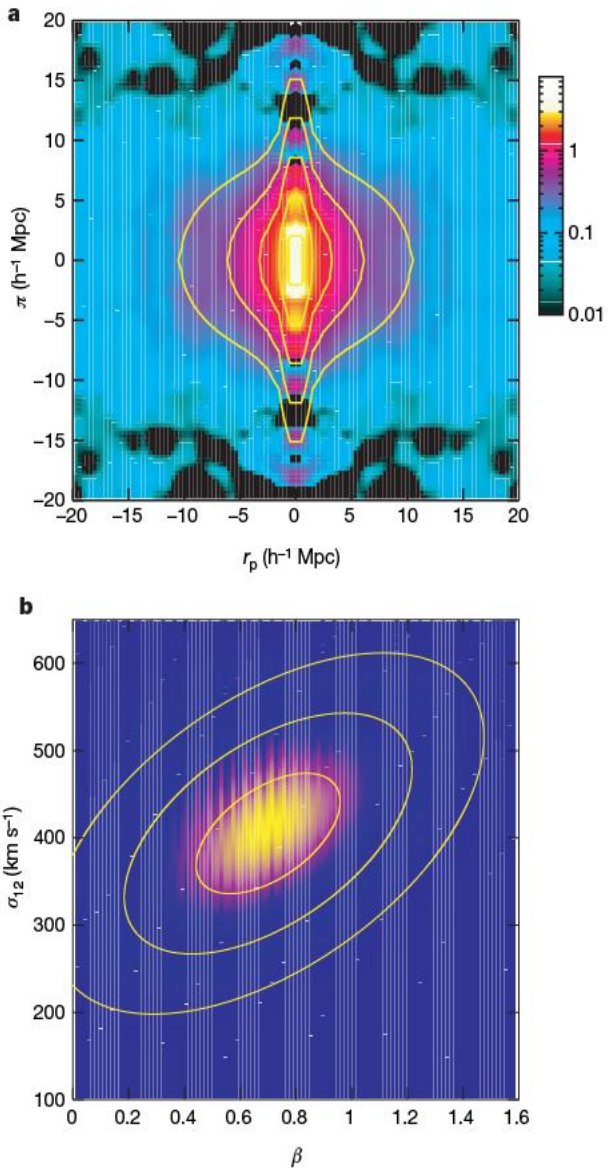

Figure 1 | Estimate of the degree of distortion induced by coherent motions on the measured large-scale distribution of galaxies at high redshift. For a given mean density of matter, this depends on the amount of dark energy and is quantified by the level of anisotropy in the galaxy correlation function $\xi(r_p,\pi)$. **a**, The colour scale represents $\xi(r_p,\pi)$ as measured using ~6,000 galaxy redshifts with $0.6 < z < 1.2$ (effective redshift $\langle z \rangle = 0.77$) in the VVDS-Wide survey. The intensity levels describe the measured degree of correlation as a function of the transverse (r_p) and radial (π) separation of galaxy pairs. $\xi(r_p,\pi)$ has been computed in pixels of $1h^{-1}$ Mpc per side and smoothed with a gaussian kernel before plotting. The actual measurement is replicated over four quadrants to show the deviations from circular symmetry. Galaxy peculiar velocities combine with the cosmological expansion, producing the distorted pattern when the redshift is used as a distance measure. In the absence of peculiar motions, the contours would be perfect circles. The effect of galaxy infall caused by the growth of large-scale structure is evident in the flattening of the purple–blue large-scale levels, while the small-scale elongation along π (white–yellow–red contours) is the result of the high-velocity-dispersion pairs in group and clusters of galaxies ('fingers of God'). The superimposed solid contours correspond to the best-fitting distortion model with a compression parameter $\beta = 0.70$ and a pairwise dispersion $\sigma_{12} = 412\,\mathrm{km\,s^{-1}}$, obtained by maximizing the model likelihood given the data. **b**, Confidence levels for the compression parameter β and the dispersion of relative velocities of galaxy pairs, σ_{12}; the contours correspond to the bivariate gaussian that best reproduces the distribution of 100 Monte Carlo measurements on fully realistic mock realizations of the data, constructed from numerical simulations (see Supplementary Information). The solid lines correspond to one-parameter confidence levels of 68%, 95% and 99%, such that marginalizing over σ_{12}, we obtain the root-mean-square uncertainty on β.

$\xi(r_p,\pi)$. Here, r_p and π are respectively the transverse and line-of-sight components of galaxy separations[17] (see Supplementary Information for definitions). The anisotropy of $\xi(r_p,\pi)$ has a characteristic shape at large r_p that depends on the parameter[18,19] $\beta = f/b_L$. In practice, we observe a compression that is proportional to the growth rate, weighted by the factor b_L, the linear bias parameter of the specific class of galaxies being analysed. b_L measures how closely galaxies trace the mass density field, and is quantified by the ratio of the root-mean-square fluctuations in the galaxy and mass distributions on linear scales[20]. Using this technique, a value of $\beta = 0.49 \pm 0.09$ has been measured at $z \approx 0.15$ using the 2dF Galaxy Redshift Survey (2dFGRS) sample of 220,000 galaxies with bias[10,21] $b_L = 1.0 \pm 0.1$, corresponding to a growth rate of $f = 0.49 \pm 0.14$.

We have measured the parameter β at an effective redshift $z = 0.77$, using new spectroscopic data from the Wide part of the VIMOS-VLT Deep Survey (VVDS)[11,12]. The redshift-space correlation function $\xi(r_p,\pi)$ has been estimated from a recently completed subset of 5,895 faint galaxy redshifts between $z = 0.6$ and $z = 1.2$, covering an area of 4 square degrees (the F22 field; see Supplementary Information for more details). This corresponds to an effective sampling volume of $6.35 \times 10^6 h^{-3}\,\mathrm{Mpc}^3$, at a median epoch of ~7 Gyr, that is, about half the age of the Universe.

$\xi(r_p,\pi)$ has been estimated in the conventional way by comparing the number of galaxy pairs at different separations (r_p,π) to that in a random sample with an identical geometry and sampling pattern (Fig. 1a). The evident ellipsoidal shape (that is, the compression of the iso-correlation contours along the line of sight—the vertical direction) is the fingerprint of galaxy streaming motions. The corresponding value of β can be measured by expanding the observed $\xi(r_p,\pi)$ in spherical harmonics; the coefficients of the expansion can be theoretically expressed as functions of β and the best value of this parameter obtained through different fitting techniques[18,19]. We have directly tested these methods on fully realistic simulations of our data (M.P. *et al.*, manuscript in preparation); we obtained the least biased and most stable results through a direct maximum-likelihood fit of the full spherical harmonic model for $\xi(r_p,\pi)$, convolved with an exponential function that accounts for the small-scale nonlinear contribution[21] (see Supplementary Information for details). This model is characterized by two free parameters, the linear compression β and the root-mean-square velocity dispersion of galaxy pairs σ_{12}, describing the small-scale incoherent motions in groups and clusters. The model that maximizes the likelihood, given our data, has $\beta = 0.70$ and $\sigma_{12} = 412\,\mathrm{km\,s^{-1}}$ (corresponding to the superimposed contours in Fig. 1a).

To estimate realistic errors for these values, we applied the same procedure to 100 independent mock replicas of our survey constructed from the Millennium simulation[22] including the full observing strategy, selection mask and redshift errors of the VVDS. These state-of-the-art simulations are highly successful in reproducing a wide range of galaxy and large-scale structure properties and can be considered in many respects as Monte Carlo realizations of our data. They allow us to include in the error budget a fair estimate of the finite sampling noise and of the 'cosmic variance' due to fluctuations on scales larger than the sampled volume. In Fig. 1b the contours correspond to the bivariate gaussian describing the distribution of the 100 mock measurements, centred on the best-fit (β,σ_{12}) pair from the data. The mean values of both parameters from the 100 mock catalogues ($\beta = 0.62 \pm 0.03$, $\sigma_{12} = 382 \pm 12\,\mathrm{km\,s^{-1}}$) are remarkably close to those measured from the data. This adds to our confidence in the overall realism of the simulations and consequently in the various tests performed to assess the robustness of our result (see Supplementary Information for details). Marginalizing over the root-mean-square pairwise dispersion (that is, integrating along σ_{12}), we obtain an estimate of the error on the compression parameter, such that $\beta = 0.70 \pm 0.26$.

Because both the growth rate and galaxy bias evolve with redshift, this value represents a mean over the redshift range $0.6 < z < 1.2$,

weighted by the radial selection function of the sample. We take the effective redshift for this measurement to be $z = 0.77$, which corresponds to the mean value of the squared redshift distribution $N^2(z)$. This is a natural choice because $\xi(r_p,\pi)$ depends on the distribution of galaxy pairs. The goodness of this choice has then been verified using the mock samples, where both the value of $\Omega_m(z)$ and $b_L(z)$ are known or can be directly recovered. In this way, the behaviour of $\beta(z) = \Omega_m^{0.55}(z)/b_L(z)$ can be directly compared to the estimated global value from the whole redshift range. This shows that our estimate of β should coincide with $\beta(z = 0.77)$ within 3%, which is well below our statistical errors (see Supplementary Information).

This is the first measurement of β at a redshift approaching unity based on a fully homogeneous galaxy redshift survey over a large volume and with accurate control over selection biases, finite sampling and cosmic variance errors. The detection and quantitative measurement of galaxy streaming motions at an epoch when the Universe was significantly younger is an important observational result in itself, testifying to the gradual growth of structure and corroborating the gravitational instability picture. To translate this measurement into an estimate of the growth rate $f = \beta b_L$, we need to know the effective linear bias factor characterizing the mean relative clustering of our galaxies with respect to the underlying mass. With sufficient statistics, b_L can be determined directly from the survey data, by measuring the higher-order details of the clustering pattern[20], but this would require a survey several times larger than that used here.

We thus adopt a different approach that has already been successfully applied to the Deep part of the VVDS survey[23]. This requires including additional information provided by independent observations, such as the level of anisotropy in the Cosmic Microwave Background[24] or the mean number density of galaxy clusters [25,26]. Both of these measurements constrain the root-mean-square amplitude of mass density fluctuations on a given scale, conventionally measured in spheres of $8h^{-1}$ Mpc radius and indicated as σ_8. The Cosmic Microwave Background data from the Wilkinson Microwave Anisotropy Probe (WMAP) experiment[24] indicate that $\sigma_8^{mass}(z = 0) = 0.78 \pm 0.03$. This allows us to estimate $b_L = \sigma_8^{gal}(z = 0.77)/\sigma_8^{mass}(z = 0.77)$. $\sigma_8^{gal}(z = 0.77)$ is measured directly from the sample by counting the number of galaxies in randomly placed spheres; the corresponding mass value is instead obtained by scaling the WMAP value to $z = 0.77$ using linear theory in a self-consistent cosmology (which has a weak influence on the final result, for a flat geometry). In this way, we obtain $b_L = 1.3 \pm 0.1$, corresponding to a growth rate of $f(z = 0.77) = \beta b_L = 0.91 \pm 0.36$.

It is interesting to compare this measurement to available model predictions (Fig. 2). These include the standard flat ($\Omega_{m0} = 0.25$, $\Omega_0 = 0.75$) cosmological-constant model, an open model with the same Ω_{m0} but no cosmological constant, the DGP braneworld modification of gravitational theory[7] and two cases in which the dark matter component interacts with the dark energy field[5]. Clearly, error bars on this measurement alone are still too large to discriminate among these models. We also show in Fig. 2 the few existing measurements of f at lower redshift. These include a value at $z \approx 0.15$ from the 2dFGRS[21] and another estimate at $z = 0.55$ that we have computed using a recent measurement of β from a survey of luminous red galaxies[27]. This value can only be taken as indicative, as it was obtained by an analysis that tries to account for extra distortions due to the geometric Alcock–Paczynski effect[28] and imposes the additional constraint of Ω_m matching the evolution of clustering to $z \approx 0$ (see Fig. 2 legend and Supplementary Information).

With these caveats in mind, it is nevertheless encouraging to observe a coherent trend in the measurements. In particular, considering the standard general theory of relativity framework, even with the current error bars the evolution of the growth rate evidently disfavours a Universe with open geometry containing only matter (at the level of ~25% of the critical density as measured by several independent probes[10,21,25,26]). This is a relevant result, as it represents an indication, independent of the Cosmic Microwave Background[24], of the need of extra dark energy to bring the curvature close to zero. We note that a purely illustrative χ^2 fit of the three data points to the functional form $f(z) = [\Omega_m(z)]^\gamma$ would indeed favour the flat cosmological constant model with growth index $\gamma \approx 0.55$–0.6, although with rather low confidence.

To discriminate among different dark energy or modified-gravity models at a finer level will require more precise estimates of β and b_L or a larger number of independent measurements with similar precision. Ongoing and planned redshift surveys are expected to fulfil this need in the near future, both in quantity and quality (see Supplementary Information). Overall, these results suggest that

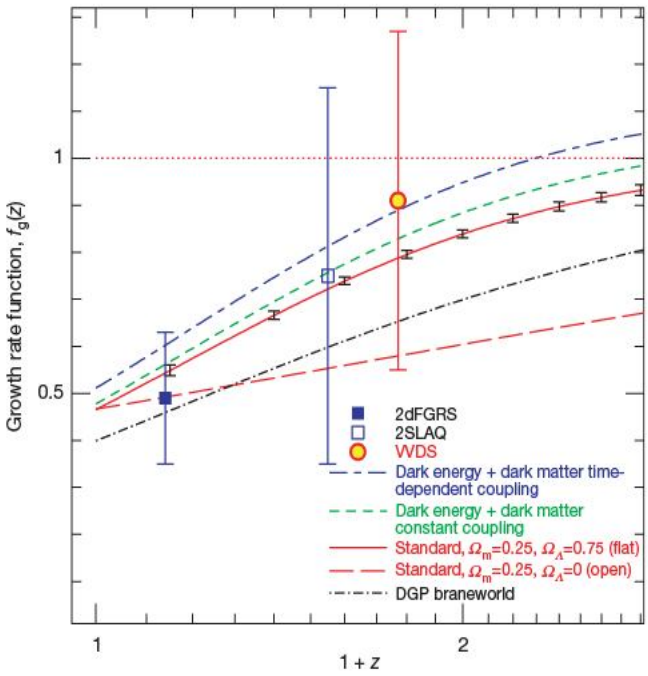

Figure 2 | Estimates of the growth rate of cosmic structure compared to predictions from various theoretical models. Values of $f = \beta b_L$ are plotted as a function of the inverse of the cosmic expansion factor $1 + z = a(t)^{-1}$. Our new measurement at $z = 0.77$ from the VVDS-Wide survey (yellow–red circle) is shown together with that from the 2dFGRS, computed from the published[21] value of β; to do this, we adopted the bias value $b_L = 1.0 \pm 0.1$ estimated from higher-order clustering in the same survey[20]. We have also used very recent measurements from the 2dF-SDSS LRG and QSO (2SLAQ) survey of luminous red galaxies[27] (blue open square) to add one further point at z = 0.55. In this case, however, the values of β and b_L are not fully independent, because they have been obtained by imposing simultaneous consistency with the clustering measured at $z = 0$. In practice, this forces the resulting f towards the flat model, that is, $\sim\Omega_m^{0.55}$. A more appropriate treatment would require an independent estimate of the bias for this sample[23]; this uncertainty is accounted for by the error bars, which in all cases correspond to 68% confidence intervals. The solid red line gives the growth rate for the standard cosmological-constant flat ($\Omega_{m0} = 0.25$, $\Omega_0 = 0.75$) model, while the dashed red line is the corresponding open model with the same matter density but no cosmological constant; the blue and green dashed curves describe models in which dark energy is coupled to dark matter[5]; the black dot-dashed line is the DGP braneworld model, an extra-dimensional modification of the gravitation theory[7]. For reference, the red horizontal dotted line $f(z) \equiv 1$ corresponds to the constant growth rate we expect in a critical-density Einstein–De Sitter model, in which the flat geometry is due to matter only ($\Omega_{m0} = 1$). Interestingly, despite the large error bars, the available measurements coherently indicate the need for a low Ω_{m0}, but at the same time disfavour an open model, thus requiring the presence of a cosmological constant or dark energy. We also provide an example of the accuracy achievable by future surveys in discriminating which kind of dark energy model is correct: the small black error bars on the standard curve (red) show forecasts for measurements in bins of size $\Delta z = 0.2$ from an all-sky survey of a half-billion infrared-selected ($H < 23$) galaxies, as recently proposed to the ESA Cosmic Vision programme by the SPACE consortium (http://urania.bo.astro.it/cimatti/space/).

redshift-space distortions will become a primary method in the quest to identify the nature of cosmic acceleration.

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LETTERS

Origin of morphotropic phase boundaries in ferroelectrics

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A piezoelectric material is one that generates a voltage in response to a mechanical strain (and vice versa). The most useful piezoelectric materials display a transition region in their composition phase diagrams, known as a morphotropic phase boundary[1,2], where the crystal structure changes abruptly and the electromechanical properties are maximal. As a result, modern piezoelectric materials for technological applications are usually complex, engineered, solid solutions, which complicates their manufacture as well as introducing complexity in the study of the microscopic origins of their properties. Here we show that even a pure compound, in this case lead titanate, can display a morphotropic phase boundary under pressure. The results are consistent with first-principles theoretical predictions[3], but show a richer phase diagram than anticipated; moreover, the predicted electromechanical coupling at the transition is larger than any known. Our results show that the high electromechanical coupling in solid solutions with lead titanate is due to tuning of the high-pressure morphotropic phase boundary in pure lead titanate to ambient pressure. We also find that complex microstructures or compositions are not necessary to obtain strong piezoelectricity. This opens the door to the possible discovery of high-performance, pure-compound electromechanical materials, which could greatly decrease costs and expand the utility of piezoelectric materials.

Originally the term 'morphotropic' referred to phase transitions due to changes in composition[1,2], but it has come to be used mainly for the common 'morphotropic phase boundaries' (MPB) that separate regions of tetragonal symmetry from those of rhombohedral symmetry by varying the composition in ferroelectrics[4]. We use the term here also to describe the same phenomenon induced by pressure. It was recently understood that the apparent continuous-phase transitions through the MPB region from tetragonal to rhombohedral, which are not allowed by symmetry, are mediated by intermediate phases of monoclinic symmetry[5], and that the high electromechanical response in this region is related to this phase transition[6] because of symmetry-allowed polarization rotation[7,8].

That the most useful ferroelectrics of this type are complex materials has complicated the discovery and development of even-higher-performance piezoelectrics. For example, relaxor single crystal ferroelectrics such as PMN-PT ($PbMg_{1/3}Nb_{2/3}O_3$-$PbTiO_3$) have electromechanical coupling ten times that of the currently used PZT ($PbZrO_3$-$PbTiO_3$) materials, and are revolutionizing applications ranging from medical ultrasound and sonar to energy harvesting. However, because the materials are complex solid solutions which melt incongruently, the melt-grown crystals are zoned, and must be selected for desired properties, a costly process that has delayed more widespread use of these promising materials and complicated the study of the physical origin of their behaviour. Such behaviour in a pure material could greatly advance the field. Here we show such behaviour in pure $PbTiO_3$ under pressure.

$PbTiO_3$ has long been considered a simple classic ferroelectric, with a single phase transition from the ferroelectric tetragonal structure to cubic perovskite at 766 K and ambient pressure[9]. A classic Raman study showed two soft modes, both vanishing at a pressure of 12 GPa at 300 K (room temperature)[10]. For the zero-temperature theoretical computations[3] to be consistent with the room-temperature data requires low-temperature phase transitions, so we performed cryogenic high pressure *in situ* Raman and synchrotron powder X-ray diffraction experiments[11,12] to explore the theoretical predictions of a series of phase transitions in $PbTiO_3$.

Figure 1 shows representative diffraction patterns measured at 10 K, indicating pressure-induced structural transitions. Energy dispersive diffraction shows only intensity changes with changing pressure whereas the high-resolution diffraction data clearly display peak splittings that result from symmetry-lowering transitions (Fig. 1 inset). The two experiments are therefore complementary and allow us to choose limited angular ranges to scan in the high-resolution measurements while ensuring complete coverage of the diffraction pattern.

Figure 2a shows our low-frequency Raman spectra and their pressure dependences are shown in Fig. 2b. There are two soft modes: one hardens above 15 GPa and the other hardens above 20 GPa. Figure 2c displays the pressure dependence of the intensity of the Raman band (centred at 190 cm^{-1}) and the inset shows the high-frequency Raman spectra. These results indicate that there are two successive phase transitions, at 15 and 20 GPa. The presence of the higher-frequency Raman modes indicates that the phase above 20 GPa is not cubic in symmetry. The appearance of a peak at 400 cm^{-1} above 20 GPa is consistent with the rotation of the TiO_6 octahedra that results in a rhombohedral $R\bar{3}c$ structure predicted in our computations and observed in PZT[13] (Supplementary Fig. 7).

The X-ray and Raman data are consistent with monoclinic symmetry between 10 and 20 GPa. Monoclinic phases, identified by similar splitting of tetragonal diffraction peaks, were also found at the morphotropic phase boundaries in PZT, PMN-PT and PZN-PT[5,14–16]. Two monoclinic phases are possible depending on the direction of the polarization. For polarizations along [*xxz*] the space group is *Cm* (designated M_A), and for polarizations along [*x*0*z*] the space group is *Pm* (designated M_C)[17]. The tetragonal (110)–(101) doublet would split into a quadruplet in both *Pm* and *Cm* monoclinic phases and revert to a doublet in the rhombohedral phase. These changes would be accompanied by a splitting of the tetragonal (111) singlet into a doublet in the monoclinic *Pm* phase as well as

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in the rhombohedral $R\bar{3}c$ phase, and a triplet in the monoclinic *Cm* phase. Our high-resolution angle-dispersive experiment performed at 115 keV (0.1077 Å) was capable of resolving a splitting of the tetragonal doublet, which points to a lowering of symmetry. We can clearly identify the tetragonal–monoclinic transition at 11.4 GPa from the splitting of the (110)–(011) doublet. The monoclinic phase persists up to 20 GPa. The persistence of the overall monoclinic symmetry is clear in the high-resolution X-ray data, although all of the peaks are not resolved clearly, owing to the resolution of the angle-dispersive experiment at the low angles accessible in the diamond anvil cell (DAC) in the cryostat. We also attribute the changes observed in the Raman spectra at 15 GPa to the *Pm*–*Cm* transition at this pressure.

We fitted our high-resolution diffraction data while fixing the peak profiles using the Au pressure standard as reference. This allowed us to model broadening due to non-hydrostatic stresses (we found no signs of stress-induced peak broadening in the pressure–temperature range of these experiments). The fits are superposed on the observed data in Fig. 1. The same fits allowed us to obtain cell parameters to a high degree of precision using a small subset of the diffraction peaks obtained from high-resolution X-ray diffraction experiments. Using these cell parameters, we calculated two lattice strains. The longitudinal strain is related to the axial ratio c/a and the shear strain to the angle $\beta - \pi/2$. The parameter c/a shows weak discontinuities across the phase transitions at 11.4 and 15 GPa, whereas the shear strain assumes a non-zero value above 11.4 GPa and monotonically decreases to zero above 20 GPa (Fig. 3). The discontinuity in cell parameters is consistent with a weak first-order transition from *Pm* to *Cm*, and is hard to explain in any other way that is consistent with our Raman data.

Using density functional theory within the local density approximation, Wu and Cohen[3] theoretically predicted a series of phase transitions in $PbTiO_3$ between tetragonal and cubic (24 GPa) as a function of pressure at 0 K. They found that the tetragonal phase is stable below 10 GPa with the lowest free energy, the monoclinic phase has the lowest free energy between 10 and 12 GPa, and the zone-centre rhombohedral phase is the stable phase above 12 GPa. Additional calculations we performed show zone-boundary transitions

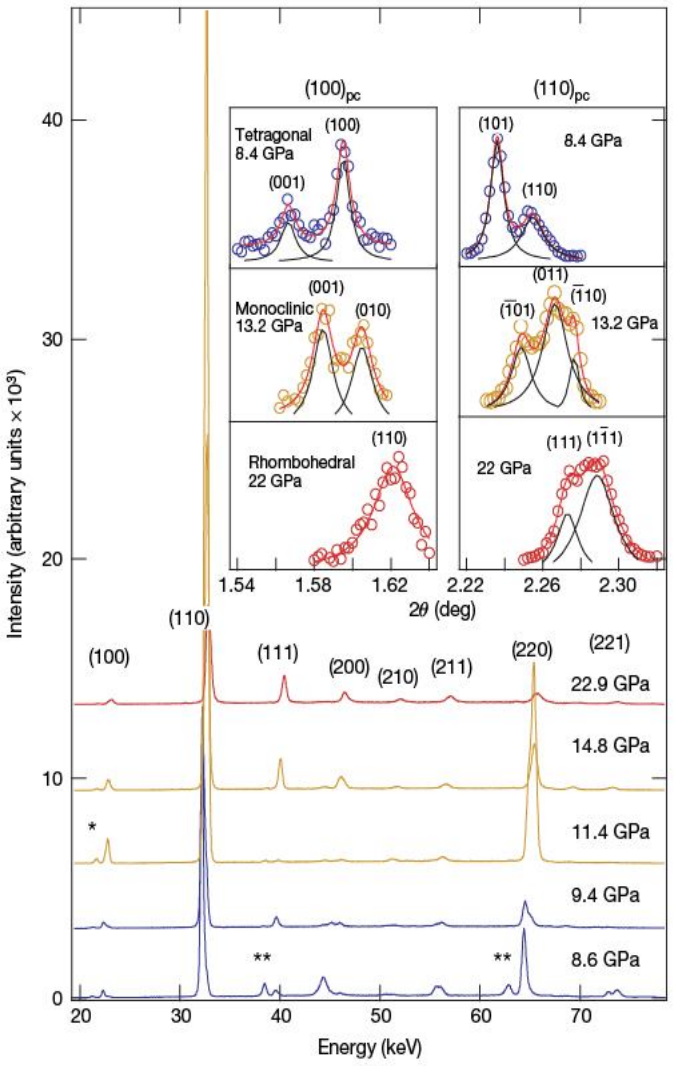

Figure 1 | Pressure dependence of energy dispersive and high-resolution angle-dispersive X-ray diffraction spectra at selected pressures at 10 K. The single asterisk represents the ghost peak and the double asterisks represent the Au peaks. The major reflection lines were indexed with a pseudocubic symmetry (pc). For instance, the pseudocubic (110) reflection splits into a doublet in the tetragonal phase and a quadruplet in the monoclinic phase. The inset shows high-resolution diffraction data at different pressures: the left panels show the pseudocubic (100) reflection at 8.4 GPa (tetragonal phase), at 13.2 GPa (monoclinic phase, the (100) reflection is missing) and at 22 GPa (rhombohedral phase); the right panels show the pseudocubic (110) reflection at 8.4 GPa (tetragonal phase), at 13.2 GPa (monoclinic phase, the (101) reflection is missing), and at 22 GPa (rhombohedral phase).

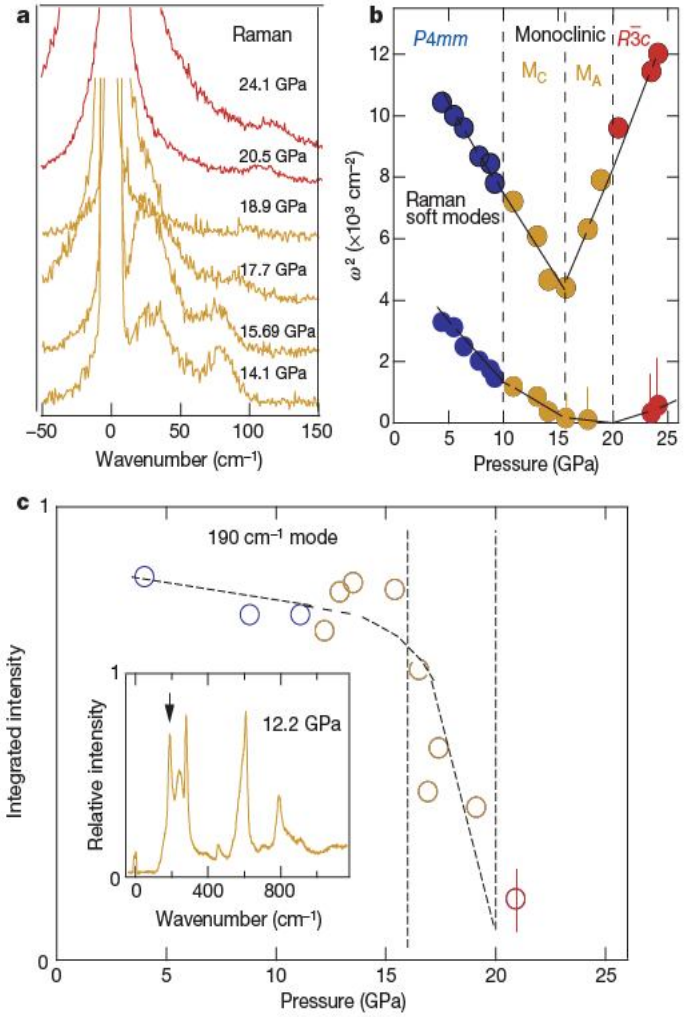

Figure 2 | Raman spectra. a, Raman spectra at selected pressures. **b**, Pressure dependences of the squares of soft-modes frequencies. Points are data and lines are guides to the eyes. The symbol size represents the size of the error bars (±50 cm^{-2}). Above 20 GPa, the Raman band becomes broad; the vertical lines represent the Raman linewidth. **c**, Pressure dependence of the integrated intensity of the Raman band centred at 190 cm^{-1} (standard error ±0.04; the largest error is ±0.125 at 20 GPa); the inset shows Raman spectra at 12.4 GPa at 20 K. The changes in soft-modes and intensity of the Raman band at 15 and 20 GPa reflect the monoclinic M_C to monoclinic M_A and the monoclinic M_A to rhombohedral phase transitions. These results indicate that the monoclinic phase transitions are real phase transitions, and are not due to nanotwinning or microstructure changes.

from $R3m$ to $R3c$ at 18 GPa, $R3c$ to $R\bar{3}c$ at 20 GPa, and $R\bar{3}c$ to $R3c$ at 60 GPa.

Our experimental results suggest a phase transition sequence from tetragonal to monoclinic at 10 GPa, monoclinic M_C to monoclinic M_A at 15 GPa, and monoclinic to rhombohedral at 20 GPa. The high-pressure rhombohedral phase is due to a zone-boundary instability as is seen theoretically (ref. 18 and our theoretical calculations). There may be a narrow wedge of the five-atom-per-cell $R3m$ structure between the monoclinic and zone boundary R point phases, which we could not resolve in our experiments. Conventional X-ray diffraction is centrosymmetric and therefore cannot differentiate whether the zone-boundary phase is $R\bar{3}c$ or $R3c$. Figure 4 shows our proposed phase diagram. The precise details of the phase diagram may also be sensitive to non-hydrostatic stresses. The details of the phase diagram are less important than the fundamental result—that there exists a morphotropic phase boundary region under pressure at cryogenic temperatures in $PbTiO_3$.

First-principles calculations show that the soft-mode potential surface has two absolute minima along the [00±1] directions at ambient pressure owing to the large lattice strain of about 6% (ref. 19). If it were not for the lattice strain, the global minima would be along the [111] directions, as in $BaTiO_3$, and there would be a rhombohedral ground state[20]. As pressure increases, theory and experiment show decreasing lattice strain, with a ground state eventually along [111] in the predicted and observed rhombohedral phases. The monoclinic phase between the rhombohedral and tetragonal phases simply arises from higher-order terms in a Taylor expansion of the energy[17], corresponding to a rotation of the polarization direction. The ease of rotating the polarization is responsible for the predicted huge electromechanical coupling in the transition region[7,8]. The high-temperature transition additionally has an order–disorder contribution, where the four (±1±11) directions are occupied in the tetragonal phase and eight (±1±1±1) directions are occupied in the cubic phase. This allows for the direct tetragonal to cubic transition observed at room temperature. The competition with octahedral rotations, common in these materials[21,22], adds complexity to the problem.

Thus we find that a MPB phase boundary region can arise in a pure material under pressure at low temperature and consequently the polarization can easily rotate between different symmetries. Our results show clearly that the classic MPB that gives rise to large electromechanical coupling does not require random fields, compositional heterogeneity, polar nanoregions, mesostructural heterogeneity, or nanodomains. The latter are effects of the soft rotational dynamics rather than the cause of high coupling. Our results demonstrate rather that the high coupling materials such as PMN-PT and PZN-PT simply compositionally tune the inherent transition observed in $PbTiO_3$ to ambient pressure. We show the possibility of finding a pure compound with a large coupling MPB-like behaviour at room pressure, which would lead to great advances in ultrasonic and other electromechanical applications.

Our results also provide strong evidence against the nanotwin theory for the monoclinic phases at MPBs, which asserts that observed monoclinic phases[5] are actually nanotwinned rhombohedral and tetragonal nanodomains[23–25]. Our first-principles calculations clearly show the ground state to be monoclinic, and we see clear evidence of tetragonal to monoclinic, monoclinic to monoclinic, and monoclinic to rhombohedral phase transitions in our micro-Raman data (Fig. 2 and Supplementary Fig. 9), which would not be present if instead there was a progressive change in microstructure with pressure. It is true that nanodomains could give apparent monoclinic diffraction, and it is true that materials at MPBs contain complex domain microtextures, but it does not logically follow that such microtextures are evidence against monoclinic symmetry. In fact, we would expect more complex microstructures for the monoclinic phase, because there are 24 possible domain orientations, compared to six for tetragonal, for example. The complex microstructures do complicate interpretation of dynamical properties, and will give extrinsic contributions to the electromechanical coupling[26].

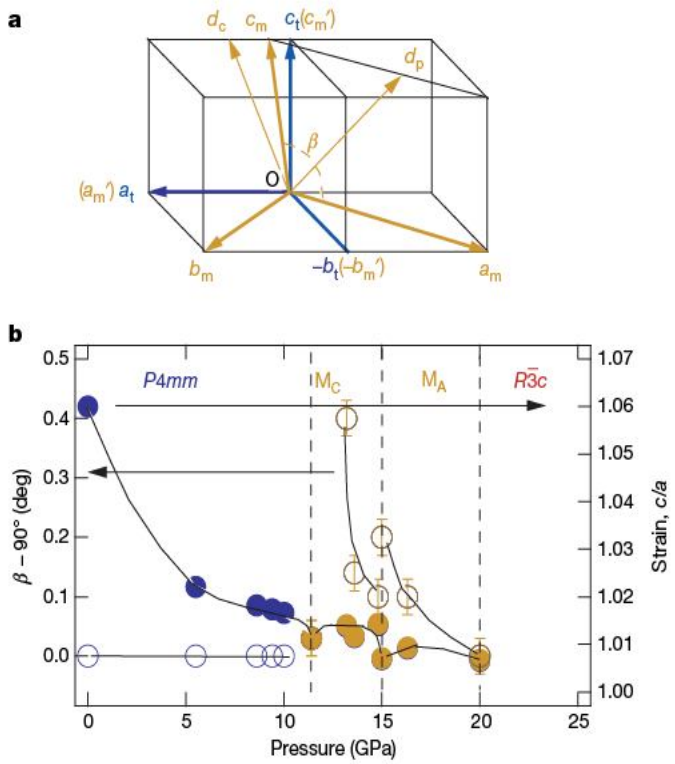

Figure 3 | Lattice strain and monoclinic angle. **a**, Representation of the monoclinic unit cell with respect to the tetragonal unit cell. The polarization in Cm (monoclinic M_A) lies in the $a_m c_m$ monoclinic plane (polarization $P = [xxz]$ in cubic cartesian coordinates) and is shown as d_p; the polarization in Pm (monoclinic M_C) lies in the $a_m' c_m'$ plane (polarization $P = [x0z]$) and is shown as d_c. **b**, Solid circles represent the pressure dependence of the c/a ratio (standard error ±0.005). The parameter c/a shows weak discontinuities across the phase transitions at 11.4 and 15 GPa. Open circles represent the monoclinic distortion angles of β–90° as a function of pressure, which corresponds to the shear strain (standard error ±0.1°). The shear strain assumes a non-zero value above 11.4 GPa and monotonically decreases to zero above 20 GPa. The phase transition point of the tetragonal T and M_C phases is slightly different in Raman and X-ray diffraction experiments. It could be that the M_C phase develops at a local scale at 10 GPa but only becomes long-range, and thus visible by X-ray diffraction, at higher pressure.

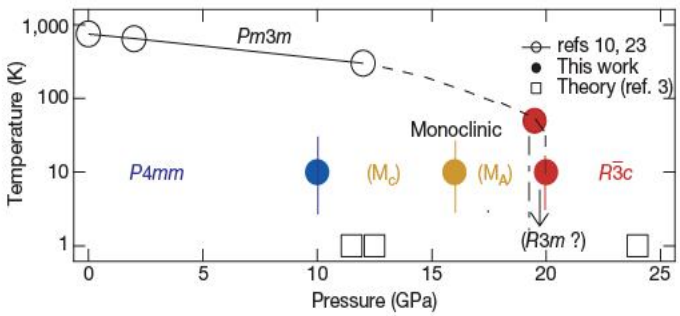

Figure 4 | Phase diagram for lead titanate. Open circles are obtained from refs 10 and 28; solid circles are experimental results from this work. Open squares are from ref. 3 (theoretical calculations), which give tetragonal symmetry below 11 GPa, monoclinic symmetry between 11 and 12 GPa, and rhombohedral symmetry above 12 GPa. The solid and dashed lines are guides for the eye and indicate the possible boundary of different phases at low temperature. Experimental results suggest that $PbTiO_3$ undergoes successive phase transitions, from tetragonal to monoclinic at 10 GPa, monoclinic M_C to monoclinic M_A at 16 GPa, and monoclinic to rhombohedral at 20 GPa. There could also be an $R3m$ phase between the M_A and $R\bar{3}c$ phases.

How can we use this information to develop new high-performance electromechanical compounds? We have shown that pressure induces large piezoelectric effects in $PbTiO_3$ in a phase transition region. The compression in the lattice constant c from 0 to 10 GPa is about 5%. We could apply chemical pressure by substituting a smaller atom with similar polarizability for Pb in the A site. Sn^{2+} has an ionic radius 6% smaller than Pb^{2+}, but tin is also less polarizable than lead. We desire an ordered compound, so we consider $Pb_{1/2}Sn_{1/2}TiO_3$ with Pb and Sn ordered in planes along 001. Local density approximation computations indeed show this to be a very promising material, with an orthorhombic ground state of symmetry $Pmm2$, (polarization along $[x0z]$, $c/a = 0.91$) with an energy difference of 12 meV per atom between $Pmm2$ and the next state Cm (polarization along $[xxx]$, $c/a = 0.98$) followed by tetragonal $P4mm$ (polarization along $[00z]$, $c/a = 1.12$) with a ΔE of 3 meV per atom. We predict strain differences $\varepsilon_{33} - \varepsilon_{11} = -0.10, -0.02$ and 0.11 in the three phases, respectively. Our results suggest ease of polarization rotation and large electromechanical strain. The properties of this compound are currently under investigation. We could design other possible materials as well from the simple idea of chemical pressure, now that we have shown the presence and origin of large electromechanical coupling under pressure in pure $PbTiO_3$.

METHODS SUMMARY

Low-temperature, high-pressure X-ray diffraction measurements on powder samples of $PbTiO_3$ were performed at synchrotron X-ray sources. Liquid He was used in flow-type cryostats to achieve low temperatures, while the samples were pressurized using the DAC. Energy dispersive X-ray diffraction experiments[11] were performed at beamline 16-BM-D of HPCAT at the Advanced Photon Source. Additional high-energy, high-resolution angular dispersive diffraction experiments[12] were performed at beamline 11-ID-C at the Advanced Photon Source. We also conducted low-temperature, high-pressure Raman scattering on the (001)-orientated single crystals of $PbTiO_3$. First-principles density functional computations were performed within the local density approximation using the ABINIT package[27].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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LETTERS

DNA-guided crystallization of colloidal nanoparticles

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Many nanometre-sized building blocks will readily assemble into macroscopic structures. If the process is accompanied by effective control over the interactions between the blocks and all entropic effects[1,2], then the resultant structures will be ordered with a precision hard to achieve with other fabrication methods. But it remains challenging to use self-assembly to design systems comprised of different types of building blocks—to realize novel magnetic, plasmonic and photonic metamaterials[3–5], for example. A conceptually simple idea for overcoming this problem is the use of 'encodable' interactions between building blocks; this can in principle be straightforwardly implemented using biomolecules[6–10]. Strategies that use DNA programmability to control the placement of nanoparticles in one and two dimensions have indeed been demonstrated[11–13]. However, our theoretical understanding of how to extend this approach to three dimensions is limited[14,15], and most experiments have yielded amorphous aggregates[16–19] and only occasionally crystallites of close-packed micrometre-sized particles[9,10]. Here, we report the formation of three-dimensional crystalline assemblies of gold nanoparticles mediated by interactions between complementary DNA molecules attached to the nanoparticles' surface. We find that the nanoparticle crystals form reversibly during heating and cooling cycles. Moreover, the body-centred-cubic lattice structure is temperature-tuneable and structurally open, with particles occupying only ~4% of the unit cell volume. We expect that our DNA-mediated crystallization approach, and the insight into DNA design requirements it has provided, will facilitate both the creation of new classes of ordered multicomponent metamaterials and the exploration of the phase behaviour of hybrid systems with addressable interactions.

Theoretical predictions indicate[15] that transitions to three-dimensional (3D) ordered phases from commonly observed disordered states[16–19] in a DNA-guided particle assembly can occur for particular shapes and ranges of interparticle interaction potentials, which are defined by the interplay of attraction and repulsion energies, E_a and E_r. The phase behaviour can be parameterized using E_a/E_r, and $\varepsilon = d_r/d_p$, which represents a relative range of repulsive interactions d_r with respect to particle size d_p (ref. 15). These parameters in DNA particle assembly systems can be conveniently controlled in a variety of ways, including the design of individual DNA, DNA shell structure and solution ionic strengths[20–23]. Experimentally, DNA-induced particle crystallization into random hexagonal close-packed crystals was observed near surfaces for single-component micrometre-sized particle systems with short-range interactions ($\varepsilon \to 0$)[9,10]. Particle crystallization with long-range interaction potentials ($\varepsilon \approx 1$) for which diverse non-close-packed structures are expected[15] (such as DNA-induced crystallization of nanoparticles) has not been achieved. Here we vary the length of the linking DNA molecules in the model DNA–nanoparticle system, providing a straightforward means of systematically changing the repulsive component of the interparticle potential.

Figure 1a and b shows a schematic illustration of the assembly system used to measure the effect of DNA structure on assembly long-range order, as studied under a variety of thermal conditions. In each assembly system, a set of DNA-capped gold nanoparticles (denoted A and B) with different DNA structures (Supplementary Table 1) were allowed to assemble by DNA hybridization into mesoscale aggregates (Fig. 1b, c). The complementary outer recognition sequences of the DNA capping provided the driving force for A and B particle assembly. The length of the recognition sequence, N_a, sets the scale of adhesion (per hybridized linker), $E_a \propto N_a$, to be from ~30 kT at room temperature (~25 °C) to ~0 kT at the DNA melting temperature T_m. Hence, E_a is approximately constant for all studied systems at any given temperature, with van der Waals interactions (<0.5 kT) contributing insignificantly[20,24]. In a "brush" regime, the length N of DNA and the flexibility of the non-complementary internal spacer allowed us to tune the range, $d_r \propto N^{3/5}$, of repulsive interaction and its strength $E_r \propto N^{3/5}/(N^{3/5} - cN_a)$, where c is defined by persistence length and molecule surface density and is constant for all studied systems[20,25]. For single-stranded DNA, experimentally relevant separations between particles, sufficient DNA surface densities and suitable salt concentrations, an estimated magnitude of E_r can reach several kT per chain[20]. Thus, the use of multiple systems with constant E_a (that is, recognition sequence), and varied d_r (that is, spacer lengths), enabled effective interparticle potential tuning, providing the environment required to achieve crystalline morphologies of nanoparticle assemblies via the thermal pathway shown in Fig. 1.

To monitor directly the *in situ* phase behaviour of the systems, we used synchrotron-based small-angle X-ray scattering (SAXS). The internal structure of the nanoparticle assemblies was investigated under different thermal conditions, including: assembly at room temperature; heating the assembly to pre-melting temperatures (T_{pm}) and DNA melting temperatures (T_m); and cooling the assembly below T_m. Figure 2 illustrates the observed scattering changes along this thermal pathway towards crystallization. The peak positions and relative heights in the SAXS patterns and in the extracted structure factors $S(q)$ (see Methods and Supplementary Discussion and Supplementary Fig 1 and 2) reveal insights into the structure of the assemblies, while the number of peaks and their widths reflect the degree of ordering within the structure.

From the thermal cycling described, we found that systems IV and V, which have the longest flexible spacers (35 and 50 bases respectively), showed spontaneous crystalline organization with remarkable degrees of long-range order. In contrast, systems with shorter spacers (systems I–III) or more rigid spacers (see Supplementary Discussion and Supplementary Fig. 1) remained amorphous upon cooling. For the crystallizing system IV (Fig. 2), the ordered structure appears after melting and subsequent cooling below T_m at ~60 °C; this is signified by the emergence of several sharp diffraction peaks in the presence of strong diffuse scattering attributed to a large contribution by the nanoparticle's form factor. This suggests the presence

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of nuclei of the newly forming phase in coexistence with unassembled particles. Upon further cooling to 59–57 °C, we observed complete crystallization of the sample, indicated by the dramatic reduction of particle form factor and the presence of sharp circular patterns characteristic of un-oriented polycrystalline samples (that is, powder scattering). This crystalline formation occurred within only a few minutes, unaffected by the cooling rate available in our set-up (up to 1 °C min^{-1}). The observed crystallization is the result of specific DNA–DNA interactions, as confirmed by multiple control experiments with noncomplementary DNA-capped nanoparticles or with uncapped particles, none of which exhibit assembly. In addition, the DNA specificity of the assembled systems is manifested in melting profiles obtained with both ultraviolet–visible spectrophotometry (Supplementary Table S2) and SAXS for all systems.

The SAXS patterns in Fig. 3 reveals seven and ten orders of resolution-limited Bragg's peaks for systems IV and V, respectively, demonstrating their crystalline 3D structures, remarkable degrees of long-range ordering, and crystallite sizes of at least ~0.5 μm, as estimated from the scattering correlation length, $\xi \approx 2\pi/\Delta q$ (ref. 26), where Δq is the resolution-corrected ($\Delta q_{res} \approx 0.0015$ Å^{-1}) full-width at half-maximum (FWHM) of the first diffraction peak. Once formed, these crystalline structures were indeed reversible, as confirmed by multiple assembly–disassembly cycles, without a noticeable loss of ordering quality or changes in system behaviour or T_m. Analysis of the peak position ratios reveal $q_x/q_1 = \sqrt{1}:\sqrt{2}:\sqrt{3}:\sqrt{4}:\sqrt{5}:\sqrt{6}:\sqrt{7}$, which correspond to the $Im'3m$ space group, a body-centred cubic (b.c.c.) structure, as shown in Fig. 3a. The $S(q)$ peak height also qualitatively follows the relative intensities predicted for b.c.c. arrangement. The observed b.c.c. structure meets the requirement for optimizing interaction energies in the studied binary AB system[15] (with AA and BB interactions being mainly repulsive), by having only particle B in the coordination shell of A and vice versa, thus forming CsCl-type superlattices.

The measured lattice parameters a for the observed b.c.c. structure are ~35 nm at 30 °C and ~42.4 nm at 28 °C for systems IV and V,

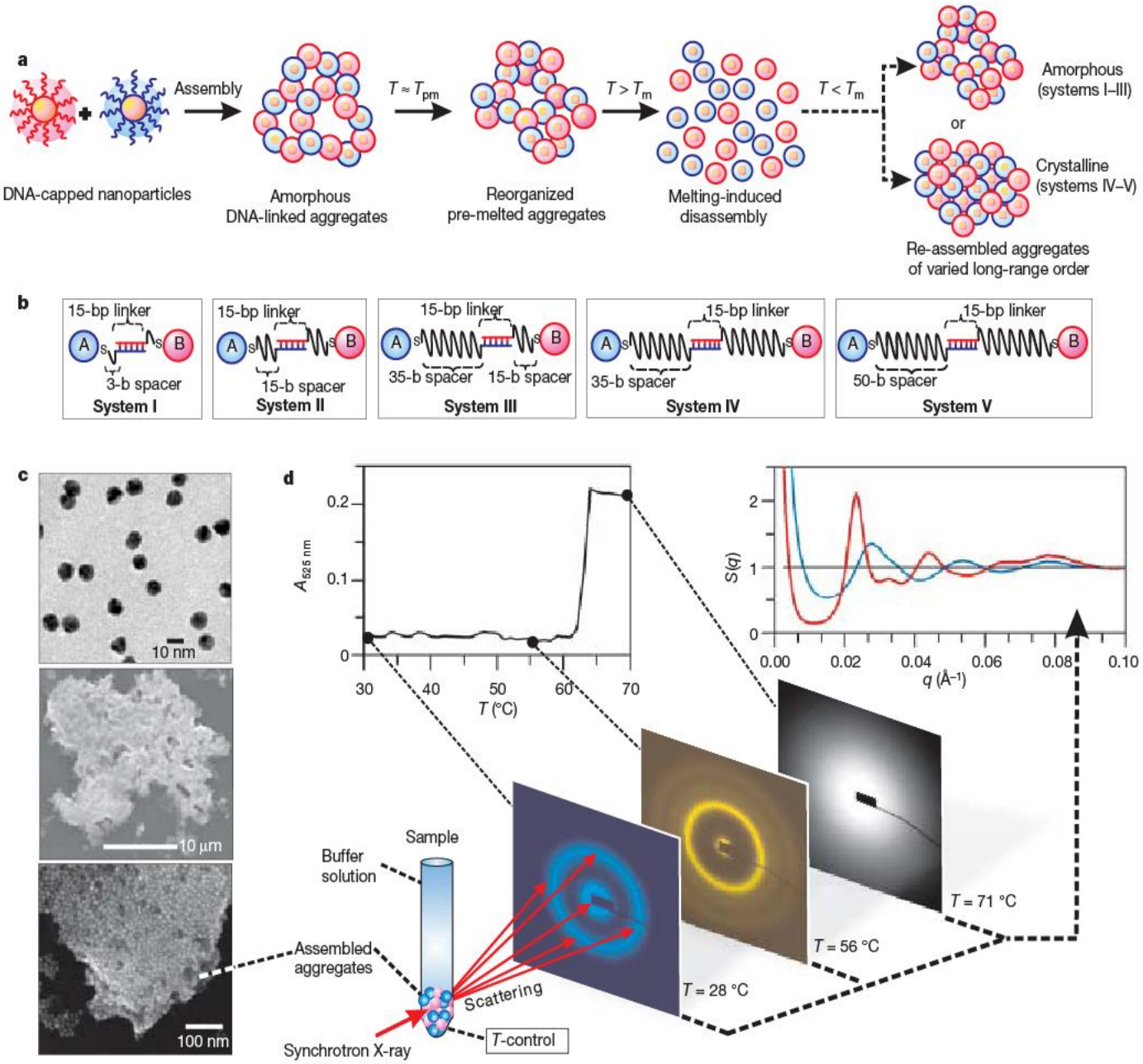

Figure 1 | Schematic of experimental design. a, The assembly system of DNA-capped nanoparticles, the aggregates of which show a series of structural changes under a variety of thermal conditions. **b**, DNA linkages between nanoparticles (one interparticle linkage is shown for clarity, not to scale) with recognition sequences for the A (blue) and B (red) sets of DNA capping. bp, base pairs. b, bases. s, thiol termination of DNA. **c**, Representative transmission (top) and scanning (middle, bottom) electron microscopy images of nanoparticles before (top) and after (middle, bottom) assembly at room temperature. **d**, Typical example of experimental measurements that reveal a correlation between the ultraviolet–visible melting profile of the aggregate and its internal structure as probed by *in situ* SAXS measurements at room temperature, pre-melting temperature, and above the disassembly/melting temperature.

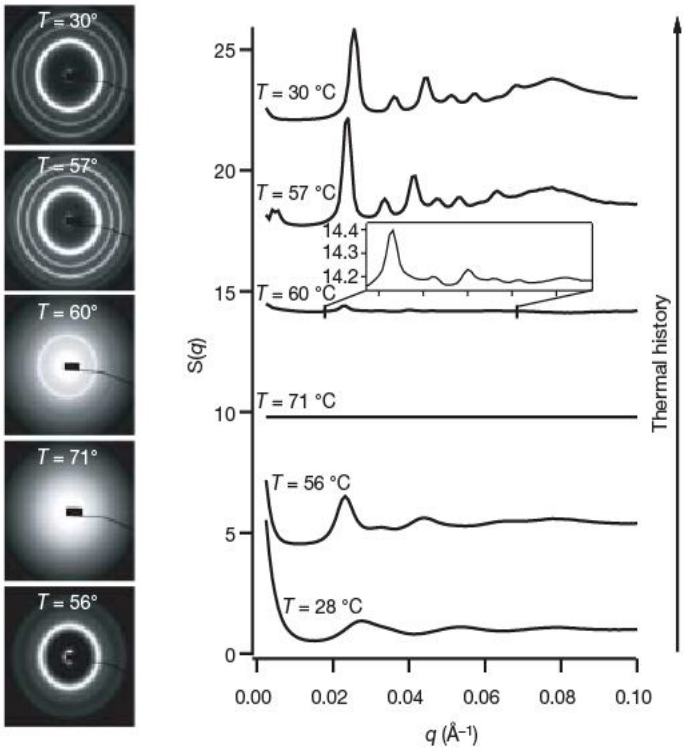

Figure 2 | Crystallization pathway for system IV. SAXS images and extracted structure factors S(q) for system IV as brought through a heating–cooling cycle for the temperatures shown (temperature stability ±0.1 °C) across the assembly melting point. S(q) lines are shifted consecutively by 4.4 units from each other. The inset shows the zoom-in area of S(q) at 60 °C for q ranging from 0.018 to 0.068 Å^{-1}.

respectively. At these a values, the particles are $d_1^{IV} = 18.9$ nm and $d_1^{V} = 24.2$ nm apart, in the first coordination shell of the obtained crystals, for systems IV and V, respectively, as calculated from a ($d_1 = \sqrt{3}/2a - d_p$). These values are close to the equilibrium linker dimensions, estimated from the scaling argument[27] for the DNA capping environment to be 18 and 23 nm. These a values indicate that the crystalline structures are remarkably open, in which nanoparticles occupy only ~2–4% of the volume and the DNAs occupy an additional 4–5%. Thus, more than ~90% of the assembled structure volume is occupied by solvent molecules, which is far higher than the typical void space in packed hard spheres in a b.c.c. orientation (~32%). Such open framework of a superlattice makes the structure vulnerable to collapse upon solvent removal, which prevents accurate morphology visualization by electron microscopy, but at the same time, makes the crystals highly accessible for modifications, molecular transport and storage. In addition, a was found to be highly sensitive to temperature and is primarily defined by thermal properties of DNA. A measured thermal expansion coefficient $\alpha \approx 3 \times 10^{-3}\,\mathrm{K}^{-1}$ (Fig. 3b, c) for systems IV and V is two orders of magnitude larger than conventional materials, promising future functional tunability at convenient temperatures.

The requirement for thermal cycling across T_m for system crystallization indicates that formation of the DNA guided nanoparticle crystals is kinetically hindered at temperatures much below T_m. The particles are probably arrested in non-equilibrium positions due to local DNA crowding and high DNA hybridization energy, exceeding 30 kT for the used recognition sequences. Structurally, this may result in non-uniaxial DNA hybridization that provides linkages at the angle to the common axes between particles, leading to possibly shorter-than-equilibrium distances between particles and distance non-uniformity (that is, amorphousness). Heating the system to T_m reduces the DNA-induced attraction energy and allows the system to anneal towards equilibrium positions. The presence of the metastable state upon initial assembly at room temperature was experimentally confirmed by sharpening of the scattering peaks, the result of more-uniform spatial organization of particles, and the increase of interparticle distances upon annealing at T_{pm} values (see Supplementary Discussion). Interestingly, this ordering with temperature is more pronounced for systems with longer linkers and more flexible spacers (for systems IV and V). These more-flexible spacer structures allow for larger local rearrangements near T_m, that

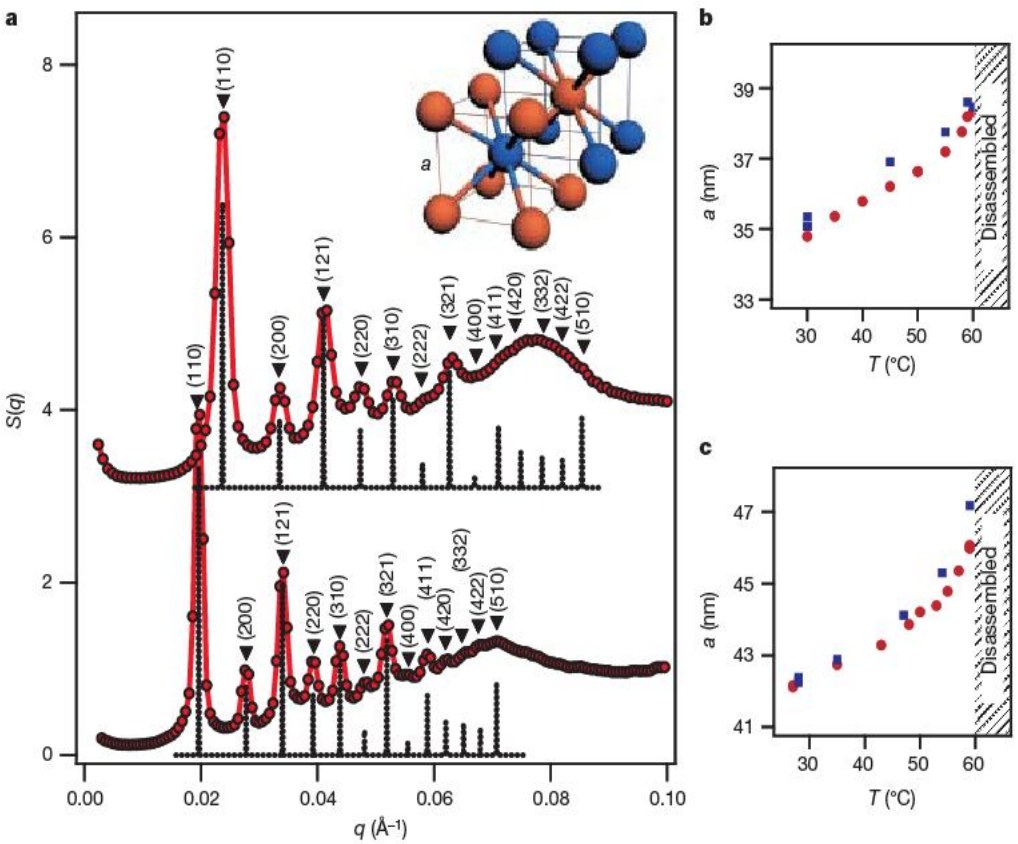

Figure 3 | Structure of crystalline DNA–nanoparticle systems. **a**, Structure factors (solid red lines with markers) for systems IV (top trace) and V (bottom trace) at 57 °C, indexed with reflections for particle arrangement in b.c.c. structure and calculated diffraction pattern (dotted line) for the b.c.c. lattice of point scatterers with corresponding lattice parameters. An illustration of the b.c.c. lattice is shown in the inset, where the proposed CsCl-type particle arrangement is coded with blue and red colours representing particles with complementary DNA cappings. **b**, **c**, Changes of lattice parameter a with temperature for systems IV and V, respectively. Red circles correspond to heating and blue squares correspond to cooling.

is, resulting in a lower energy penalty for deformation of DNA with a longer spacer by a given length, owing to the energy scaling with DNA size as $\sim(1-1/(bN^{3/5}))^{5/2}$, where b is a constant for all described systems and is determined by chain stiffness and DNA surface density[27,28]. In addition to facilitating pre-ordering below T_m, this lower penalty for DNA deformation helps particles 'squeezing' between neighbours to achieve optimal crystalline packing once bonds between particles become reversible at T_m.

The equilibrium state in the assembled system is also affected by linker structure as described above, through E_a/E_r and ε parameters. A quantitative comparison between theoretically predicted behaviour[15] and observed crystallization is problematic owing to the experimentally different realization of repulsion tuning, the influence of increased particle curvature on DNA behaviour, and effects associated with high local density of DNA chains. But qualitatively, the observed transition from disorder to b.c.c. phase occurs with increasing E_a/E_r parameter and ε close to unity—that is, at conditions similar to those predicted.

Our results show that DNA design has profound effects on the equilibrium state of the assembled systems, as well as on the kinetically favourable path to equilibrium. The proper equilibrium balance of repulsion and attraction energies of the system provides the possibility of forming ordered structures, and a smooth energy landscape, brought about by long flexible linkers and the described thermal pathway, provides the means to achieve this part of a phase space.

The thermodynamic stability and reversibility of the ordered assemblies, combined with the sensitivity of interparticle distances to biomolecular conformations and temperature, opens up new horizons for sensing, studies on biological interactions, and the tuneable functionality of metamaterials. Moreover, decoupling the assembly structure from the properties of nanoparticle constituents will allow for the incorporation of a broad variety of nanoscale objects in ordered 3D hybrid assemblies, the phases and behaviours of which are yet to be explored.

METHODS SUMMARY

Gold nanoparticles (11.4 ± 1.0 nm for systems I to IV and 12.5 ± 1.1 nm for system V) were synthesized as reported recently[16,17], and functionalized with a single-stranded DNA using methods to achieve high DNA coverage[29]. Thiol-modified single-stranded oligonucleotides (100–300 nmoles) were purchased as disulphides and reduced for 30 min with 0.1–0.3 ml of a 100 mM dithiothreitol solution in purified water or buffer before being purified with Sephadex columns. The total number of single-stranded DNAs bound to each particle was determined as $\sim 60(\pm 5)$ (~ 31.7 pmol cm^{-2}; ref. 30).

Particle assembly was carried out at 25 °C by combining equimolar amounts of type-A and type-B DNA-capped gold particles in 200 µl ([A] = [B] = ~30 nM) solution of 10 mM phosphate buffer, 0.2 M NaCl, pH = 7.1. The particles were allowed to assemble into aggregates overnight, and the resulting precipitate was collected and transferred in buffer to a quartz capillary (1.0 mm diameter), and sealed with wax.

SAXS experiments were performed at the National Synchrotron Light Source's X-21 beamline. The scattering data were collected with a charge-coupled device (CCD) area detector at wavelength $\lambda = 1.5498$ Å. The data are presented as the structure factor $S(q)$ versus scattering vector, $q = (4\pi/\lambda)\sin(\theta/2)$, where θ is the scattering angle. The values of q were calibrated with silver behenate ($q = 0.1076$ Å^{-1}). $S(q)$ was calculated as $I_a(q)/I_p(q)$, where $I_a(q)$ and $I_p(q)$ are background-corrected angular averaged one-dimensional scattering intensities for a system under consideration and un-aggregated system, respectively. The peak positions in $S(q)$ are determined by fitting a lorenzian form.

Ultraviolet–visible spectrophotometry spectra were collected on a Perkin Elmer Lambda 35 spectrometer. Melting analysis was performed in conjunction with a Perkin Elmer PTP-1 Peltier Temperature Programmer between 20–75 °C with a temperature ramp of 1 °C per min while stirring, in a solution of 10 mM phosphate buffer, 0.20 M NaCl, at pH = 7.1.

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LETTERS

DNA-programmable nanoparticle crystallization

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It was first shown[1,2] more than ten years ago that DNA oligonucleotides can be attached to gold nanoparticles rationally to direct the formation of larger assemblies. Since then, oligonucleotide-functionalized nanoparticles have been developed into powerful diagnostic tools[3,4] for nucleic acids and proteins, and into intracellular probes[5] and gene regulators[6]. In contrast, the conceptually simple yet powerful idea that functionalized nanoparticles might serve as basic building blocks that can be rationally assembled through programmable base-pairing interactions into highly ordered macroscopic materials remains poorly developed. So far, the approach has mainly resulted in polymerization, with modest control over the placement of, the periodicity in, and the distance between particles within the assembled material. That is, most of the materials obtained thus far are best classified as amorphous polymers[7–16], although a few examples of colloidal crystal formation exist[8,16]. Here, we demonstrate that DNA can be used to control the crystallization of nanoparticle–oligonucleotide conjugates to the extent that different DNA sequences guide the assembly of the same type of inorganic nanoparticle into different crystalline states. We show that the choice of DNA sequences attached to the nanoparticle building blocks, the DNA linking molecules and the absence or presence of a non-bonding single-base flexor can be adjusted so that gold nanoparticles assemble into micrometre-sized face-centred-cubic or body-centred-cubic crystal structures. Our findings thus clearly demonstrate that synthetically programmable colloidal crystallization is possible, and that a single-component system can be directed to form different structures.

From a surface receptor standpoint, gold nanoparticles can be programmed to behave as a single-component or binary system by using the sequence-specific recognition properties of DNA (Fig. 1a) and designing DNA linkers with two different regions (Fig. 1b and c). In a typical experiment, gold nanoparticles (15 nm in diameter) are modified with synthetic oligonucleotides[17] and then linker DNA is introduced; the latter contains a region 1 complementary to the

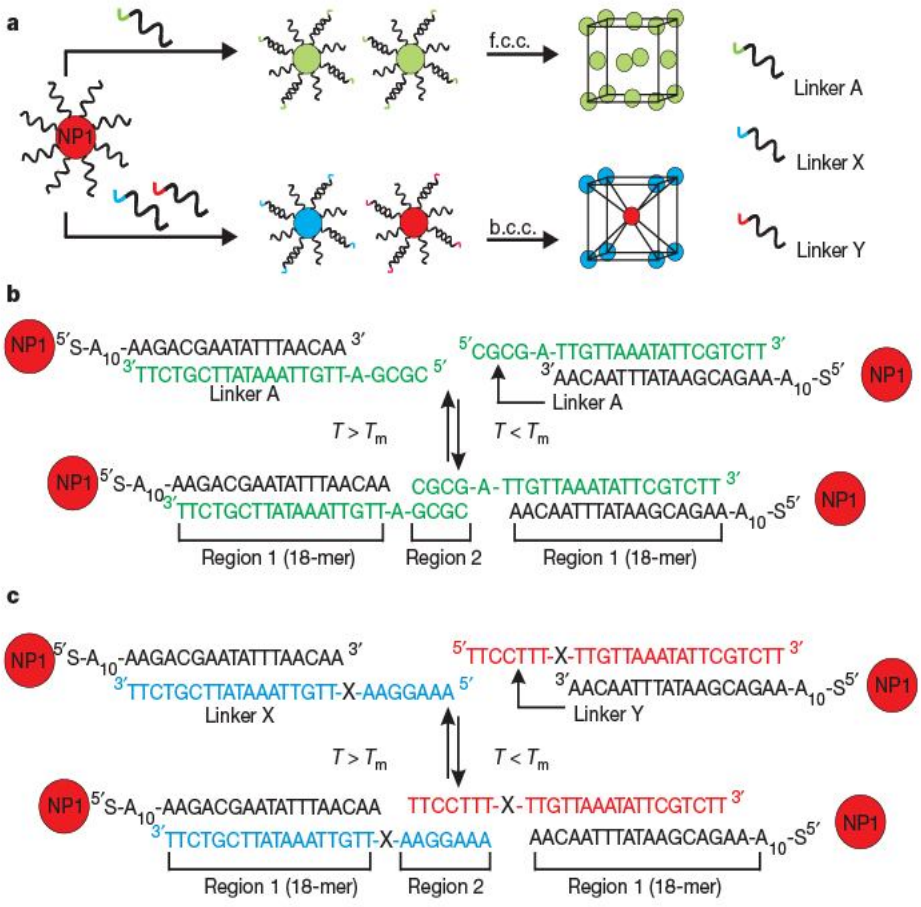

Figure 1 | Scheme of gold nanoparticle assembly method. **a**, Gold nanoparticle–DNA conjugates can be programmed to assemble into different crystallographic arrangements by changing the sequence of the DNA linkers. **b**, Single-component assembly system (f.c.c.) where gold nanoparticles are assembled using one DNA sequence, linker-A. **c**, Binary-component assembly system (b.c.c.) in which gold nanoparticles are assembled using two different DNA linkers -X and -Y. X in the DNA sequence denotes the flexor region: A, PEG_6 or no base. NP1 indicates that the same gold nanoparticle-DNA conjugates were used in all experiments.

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gold-nanoparticle-bound DNA, and a region 2 that acts as a dangling end and can be varied to control the interactions between the gold nanoparticles. In all cases, region 1 is significantly longer than region 2, and therefore the duplex formed from hybridization with region 1 is more stable than the duplex formed from hybridization with region 2. This allows region 2 to be thermally addressable without significantly perturbing region 1 (ref. 18). By designing a linker sequence in which region 2 is self-complementary, the nanoparticles will effectively behave as a single-component system (Fig. 1b). Alternatively, by designing a linker with a non-self-complementary region 2, an additional, different linker is required to achieve particle assembly (Fig. 1c). From a surface receptor standpoint, the latter design creates a binary system in which gold nanoparticles hybridized to linker-X (AuNP-X) can only bind to gold nanoparticles hybridized to linker-Y (AuNP-Y). Between region 1 and 2, a non-binding single DNA base, called a flexor, is added (typically adenosine, A). As discussed later, the flexor plays a crucial role in DNA-programmable nanoparticle crystallization.

The ability to simulate a single-component or binary system without the irreversible chemical alteration of the gold nanoparticle–oligonucleotide conjugate is a unique aspect of this system. From an energy minimization standpoint, it is expected that the gold nanoparticle assemblies will maximize the number of hybridized DNA linkages by adopting a conformation that will maximize the number of nanoparticle nearest neighbours. In a single-component system, in which each particle can bind to every other particle with equal affinity, a close-packed face-centred-cubic (f.c.c.) structure is expected to form wherein each particle has 12 nearest neighbours. Alternatively, in a binary system, where AuNP-X can bind only to AuNP-Y, the maximum number of hybridization events is achieved in a non-close-packed body-centred-cubic (b.c.c.) structure wherein each particle has eight nearest neighbours (that is, a caesium chloride lattice). Should a binary system assemble into a close-packed structure, each particle will have, on average, less than eight compatible nearest neighbours through which DNA hybridization can occur (see also Supplementary Information).

We begin by demonstrating the ability to form close-packed macroscopic single-crystalline domains using the single-component nanoparticle system (Fig. 1b). To create a well-defined and close-packed crystal, weak and reversible interactions are necessary[13,19–23]. This is achieved by combining the gold nanoparticles and linker-A above the melting temperature (T_m) of region 2 (~44 °C) followed by slow cooling (10 min/1 °C) to room temperature, to ensure that crystal formation is thermodynamically and not kinetically controlled. Two-dimensional small-angle X-ray scattering (SAXS) data collected from the resultant particle assemblies display a scattering pattern specific to a f.c.c. structure (Fig. 2a). In addition to well-defined scattering rings, individual scattering spots are clearly seen in the first and second ring indicating the formation of many large crystallites (Fig. 2b).

The majority of the high-intensity spots reside in the first ring and display nearly identical q values. The two-dimensional data were integrated and normalized based on the q value from the first ring (red line in Fig. 2c). The normalized spot positions were located at $q/q_0 \approx 1$, $\sqrt{4/3}$, $\sqrt{8/3}$, $\sqrt{11/3}$ and 2, identifying the crystalline domains as possessing f.c.c. structure when compared to the theoretical spectrum (green line in Fig. 2c). Indeed, the averaged structure factor $S(q)$ (red line in Fig. 2c) is very similar to a theoretical simulation (blue line in Fig. 2c) of the SAXS pattern for a f.c.c. configuration containing a small amount of disorder[24] (Supplementary Information). We note that the experimental data exhibit a small feature at $q/q_0 = \sqrt{3}$, which could be due to a hexagonal close-packed crystalline domain, a compressed f.c.c. crystalline domain, or a less ordered random hexagonal close-packed domain. However, the dominant overall structure is clearly f.c.c. We use the Scherrer formula[25] to estimate from the spots in the scattering pattern a size of around 2.1 μm (~10^6 particles) for the single-crystal domains. The average interparticle distance can also be determined from the position of the first peak, which in q space is placed at $\sqrt{6}\pi/d_{Au}$, where d_{Au} is the distance between the nanoparticle centres. This gives a measured interparticle distance of 27.9 nm, which falls within the range of the predicted interparticle distance (28.6–36.1 nm) that is based on the length of the DNA linkers (Supplementary Information).

More interesting than the formation of a close-packed structure is the ability to program the assembly of the same nanoparticles into a non-close-packed structure. This was achieved by using linkers -X and -Y, to create a binary system (from the surface receptor standpoint) which drives the gold nanoparticle assembly into a non-close-packed b.c.c. structure to maximize the number of DNA hybridization events (Fig. 1c). The two-dimensional SAXS pattern for this system clearly indicates a b.c.c. structure (Fig. 3a and b). The averaged $S(q)$, determined by integration and normalization based on the q value from the first ring, shows five peak positions at $q/q_0 = 1$, $\sqrt{2}$, $\sqrt{3}$, 2 and $\sqrt{5}$, in agreement with the theoretical b.c.c. structure (green line in Fig. 3b). In addition to the peak

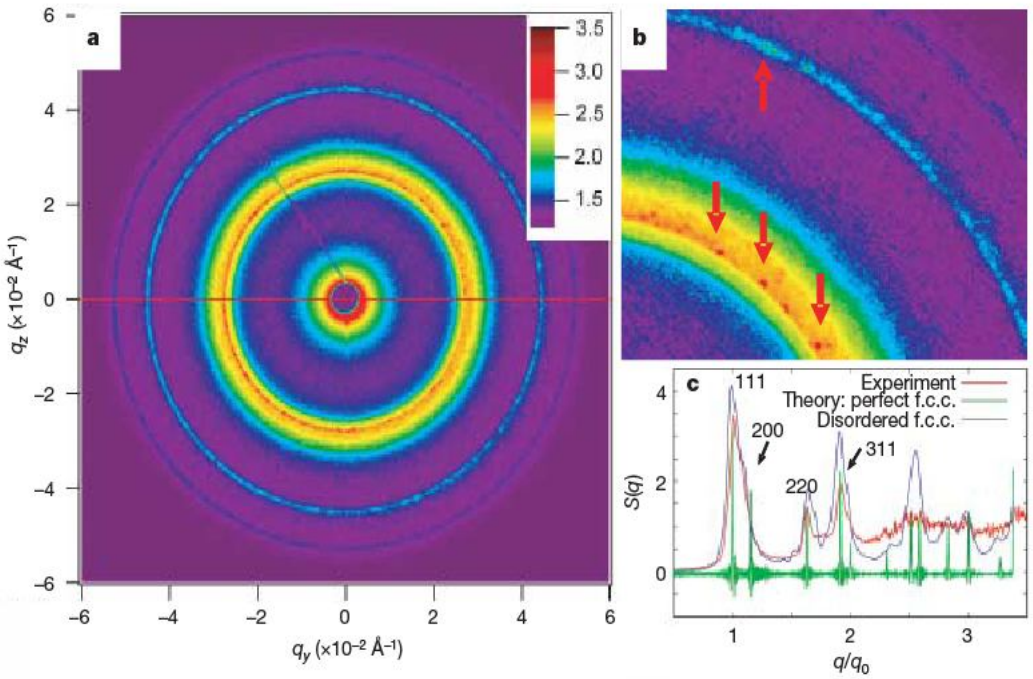

Figure 2 | f.c.c. gold nanoparticle SAXS pattern. **a**, SAXS pattern of micrometre-size single-crystalline domains using a single-component system. The colour scale indicates the intensity, I. The image is in log scale. **b**, A partial magnification of **a** displays individual spots of increased scattering intensity (red arrows). **c**, The integrated data from **a** shows an f.c.c. crystal structure. The x-axis is normalized to the first peak from **a** (2.76×10^{-2} Å^{-1}). The entire spectrum from **c** is not shown in **a**.

position, the relative peak heights are consistent with the theoretical calculations. Using the Scherrer formula, the average size of a single-crystalline domain was estimated to be about 600 nm (~10^4 particles) with an average *d*-spacing of 31 nm (estimated range 29.6–37.1 nm; Supplementary Information).

The binary system discussed above can also form a close-packed structure, by carefully controlling the temperature at which AuNP-X and AuNP-Y are combined. If the binary particles are treated in the same manner as the single-component system by combining AuNP-X and AuNP-Y above the T_m of region 2 (weak DNA attractive forces) followed by slow cooling, a substitutionally disordered f.c.c. structure[21] is formed, which presents an f.c.c. scattering pattern (Supplementary Information). Alternatively, a non-close-packed b.c.c. structure is achieved by combining AuNP-X and AuNP-Y at room temperature, below the T_m (~37 °C) of region 2 (stronger DNA attractive forces) followed by annealing a few degrees below the T_m.

The formation of the different crystal structures is attributed to a competition between the entropic and enthalpic contributions involved in the assembly process at different temperatures. From an entropic standpoint, a close-packed structure is favoured over a non-close-packed structure because the entropy of the entire system can be maximized if the aggregates possess the smallest possible volume fraction[22,26]. Therefore, if gold nanoparticles begin to assemble near the DNA T_m, where the DNA binding strength is very weak and the enthalpic contribution is small, the entropic contribution will dominate the assembly process and a close-packed structure forms. However, if the gold nanoparticles are combined several degrees below the T_m, the enthalpic contribution associated with DNA hybridization will govern the assembly process and a non-close-packed structure forms that maximizes the number of DNA hybridization events.

Gold nanoparticle–oligonucleotide conjugate systems have many variables that can be adjusted to affect the final structure. In addition to the DNA sequence as addressed above, DNA rigidity, DNA length and particle size can be manipulated to influence gold nanoparticle crystallization without changing the basic properties of the overall system. To probe the importance of these variables in the crystallization process, we began by changing the rigidity of the flexor region which is not involved in the DNA hybridization. Two variations of linker-X and -Y DNA were synthesized; one without the A-flexor and one with a polyethylene glycol oligomer (PEG_6) in place of the A-flexor (Fig. 1c). The absence of the A-flexor should result in a rigid system while the PEG_6-flexor should give a more flexible system. The first peak in the SAXS pattern from the sample with the PEG_6-flexor is the sharpest, and the sample with no flexor is the broadest, Fig. 3c. This indicates that the sample with the flexible PEG_6-flexor can grow larger crystals. Also, after similar crystallization times, a more well-defined crystalline structure arises from the PEG_6-flexor (Fig. 3d). Hence, greater flexibility can enhance the assembly process and results in a more well-defined crystalline structure.

Next, the effect of DNA length was interrogated by designing a gold nanoparticle with a shorter DNA sequence in region 1 (12-mer versus 18-mer in Fig. 4a) while maintaining an elevated T_m compared to region 2. When both AuNP-X and AuNP-Y contain the shorter region 1 DNA sequence, the result is a b.c.c. structure, similar to before, only with a shorter interparticle distance. However, the combination of a short region 1 AuNP-X and a long region 1 AuNP-Y (Fig. 4b), results in a b.c.c. structure that is thermally more stable (Supplementary Information). This suggests that the aspect ratio between the effective radii of the binary DNA-linked gold nanoparticles is an important factor in the crystallization process. As the aspect ratio of the particles decreases from one, a b.c.c. structure becomes entropically more favoured because the volume fraction of the b.c.c. structure is reduced[22,26] and the f.c.c. structure loses its entropic advantage as the particles become effectively polydisperse[27]. This was further addressed by using small gold nanoparticles (10 nm) with a short region 1 and large gold nanoparticles (15 nm) with a long

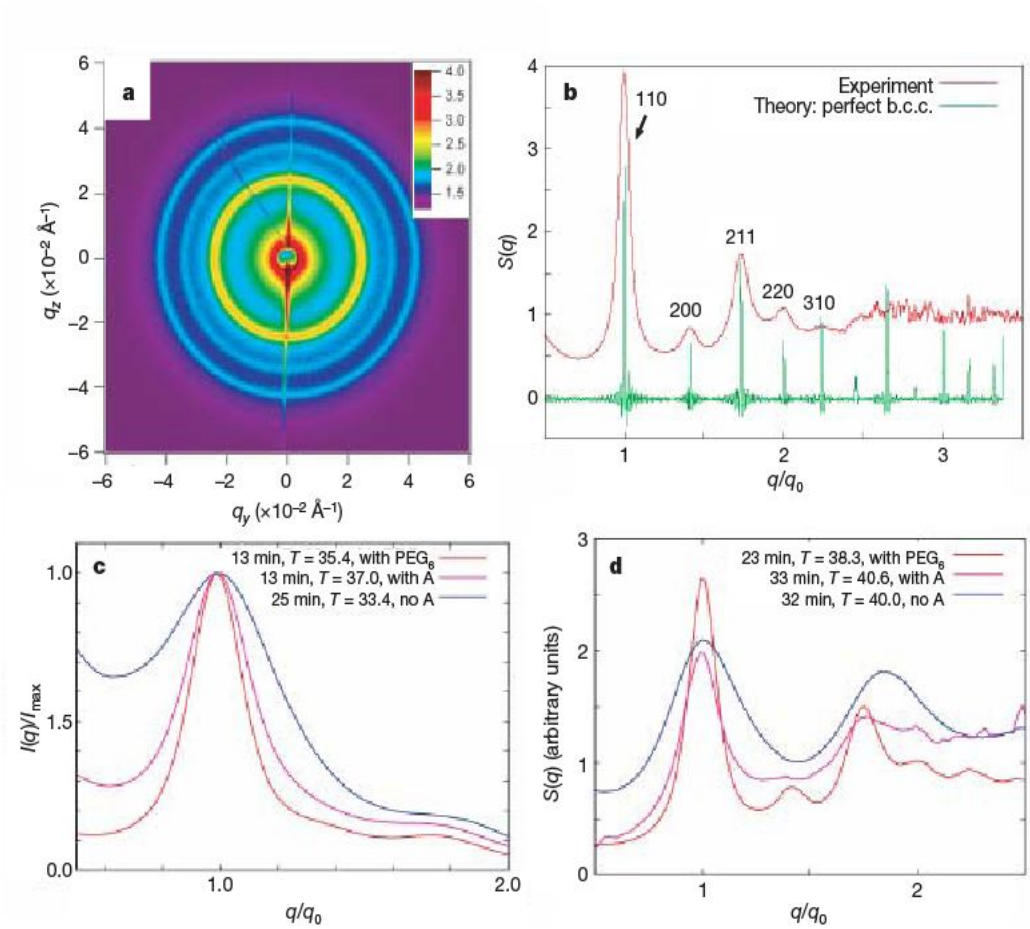

Figure 3 | b.c.c. gold nanoparticle SAXS pattern. **a**, SAXS pattern of the binary gold nanoparticle system combined below the T_m of region 2. The colour scale indicates the intensity, *I*. The image is in log scale. **b**, The integrated SAXS data from **a** shows a b.c.c. crystal structure. **c**, Comparison of the first peak between three different binary samples containing different flexor regions as assembly is initiated. **d**, Comparison of the entire SAXS pattern of three different binary samples containing different flexors (A, PEG_6, no flexor). The times given indicate the time passed since the initiation of the experiment.

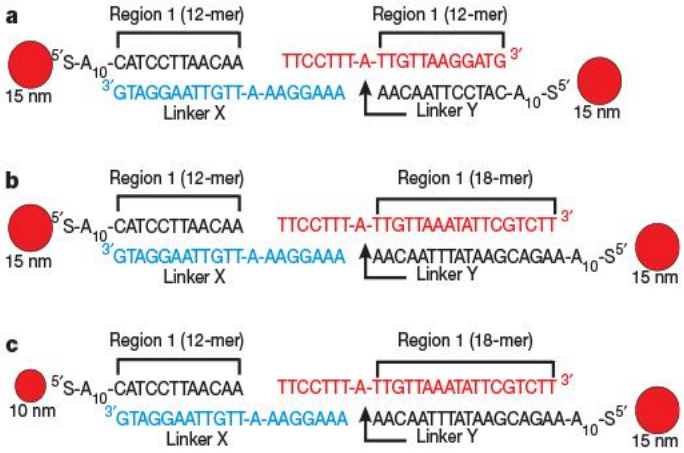

Figure 4 | Changing DNA length and gold nanoparticle size in the binary-component assembly scheme (b.c.c.). **a**, Both AuNP-X and AuNP-Y contain a shorter DNA length in region 1: 12-mer versus 18-mer from Fig. 1. **b**, Asymmetric binary gold nanoparticle assembly in which AuNP-X contains a short 12-mer region 1 and AuNP-Y contains a long 18-mer region 1. **c**, Asymmetric binary gold nanoparticle assembly in which AuNP-X in **b** is a small 10 nm gold nanoparticle and AuNP-Y is a larger 15 nm gold nanoparticle.

region 1 (Fig. 4c). These samples formed crystals with a b.c.c. structure that exhibit even greater stability, such that the b.c.c. structure can be achieved independent of pathway (that is, slow cooling from above T_m versus combining and annealing below T_m) (Supplementary Information).

In all cases, to create well-defined programmable crystalline structures using DNA-linked gold nanoparticles, several conditions must be met. In addition to having control over the strength of the DNA attractive forces, it is important to have highly monodisperse particles (<10%). As shown in a dissipative particle dynamics (DPD) simulation[28,29], particles with a polydispersity of 20% do not form well-defined crystalline assemblies, which is in accordance with our experiments (Supplementary Information). Therefore, all crystalline structures presented in this report were obtained using nanoparticles with polydispersity less than 10%.

These findings demonstrate that DNA-directed assembly affords powerful and versatile control over the formation of colloidal nanoparticle crystals. We expect that as advances in building valency into nanoparticle structures through edge- and face-selective modification processes mature[18,30], the number and type of crystalline structures accessible through this approach should significantly increase.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions C.A.M. was the originator of the concept of programmable colloidal crystallization with DNA. A.K.R.L.-J. and C.A.M were responsible for the synthetic components of the project and sequence design. S.Y.P. and G.C.S. were responsible for the theoretical components of the project. S.W. designed the SAXS set-up. S.Y.P., A.K.R.L.-J. and B.L. designed and performed SAXS experiments. S.Y.P. and B.L. analysed the SAXS data. All authors contributed to the writing of the manuscript.

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LETTERS

Large contribution of sea surface warming to recent increase in Atlantic hurricane activity

Mark A. Saunders[1] & Adam S. Lea[1]

Atlantic hurricane activity has increased significantly since 1995 (refs 1–4), but the underlying causes of this increase remain uncertain[2,5–15]. It is widely thought that rising Atlantic sea surface temperatures have had a role in this[16,17], but the magnitude of this contribution is not known. Here we quantify this contribution for storms that formed in the tropical North Atlantic, Caribbean Sea and Gulf of Mexico; these regions together account for most of the hurricanes that make landfall in the United States. We show that a statistical model based on two environmental variables—local sea surface temperature and an atmospheric wind field—can replicate a large proportion of the variance in tropical Atlantic hurricane frequency and activity between 1965 and 2005. We then remove the influence of the atmospheric wind field to assess the contribution of sea surface temperature. Our results indicate that the sensitivity of tropical Atlantic hurricane activity to August–September sea surface temperature over the period we consider is such that a 0.5 °C increase in sea surface temperature is associated with a ~40% increase in hurricane frequency and activity. The results also indicate that local sea surface warming was responsible for ~40% of the increase in hurricane activity relative to the 1950–2000 average between 1996 and 2005. Our analysis does not identify whether warming induced by greenhouse gases contributed to the increase in hurricane activity, but the ability of climate models to reproduce the observed relationship between hurricanes and sea surface temperature will serve as a useful means of assessing whether they are likely to provide reliable projections of future changes in Atlantic hurricane activity.

North Atlantic hurricane activity has increased significantly since the 1970s and 1980s. The proportion of years with activity (based on the Accumulated Cyclone Energy (ACE) index[18]) above the 1950–2000 mean has increased from 16% for the period 1970–1994 to 82% for the period 1995–2005 (ref. 4). This upswing in hurricane activity has been interpreted as being due either to a return to positive phase conditions of the Atlantic Multidecadal Oscillation[5,8,13,14] or as part of a rising trend linked to global warming[2,10–12,15]. The former cause would imply a return to below-norm hurricane activity in ~20 years, whereas the latter would suggest that hurricane activity may continue rising through the twenty-first century. To provide informed projections for future hurricane activity it is essential first to quantify the separate contributions of dynamical (that is, related to atmosphere circulation) and thermodynamical (related to sea warming) changes in the recent increase in Atlantic hurricane activity. Simulations of Atlantic hurricane activity have not yet been able to provide this information[19–22]. We deduce the contribution of sea warming to the recent rise in hurricane activity by using a statistical model.

The following data sets and procedures are employed. We use maximum sustained windspeed data from the US National Hurricane Center's North Atlantic hurricane database[23] between 1950 and 2005. As recommended by Landsea[3], no bias-removal scheme is applied to records between 1950 and 1969. We employ monthly sea surface temperature (SST) data and monthly wind records at 925 hPa (about 750 m above sea level), 850 hPa and 200 hPa between 1965 and 2005 from the National Center for Environmental Prediction/National Center for Atmospheric Research reanalysis[24]. Throughout, we define 'tropical Atlantic activity' as including those storms that form as tropical depressions within the tropical North Atlantic south of 20.0° N, the Caribbean Sea or the Gulf of Mexico. These storms account for 85–90% of the hurricanes and intense hurricanes that made landfall on the United States between 1950 and 2005. We employ this subset because statistical models best explain its interannual activity, so the uncertainties in conclusions are smaller. However, our results are robust if tropical storms from the whole North Atlantic are included (see Supplementary Information). The four standard measures of hurricane frequency and activity considered here are numbers of tropical storms, numbers of hurricanes, numbers of intense hurricanes and the ACE index.

Figure 1 compares the tropical Atlantic and US-landfalling hurricane frequency and activity between 1996 and 2005 with the long-term norm and with the previous peak in activity in the 1950s. Tropical cyclone records for the Atlantic basin are considered reliable from the mid-1960s but between 1950 and ~1965 are probably missing storms because of a lack of geostationary satellite imagery[3,25]. Activity levels are displayed as ten-year running averages, with the last value corresponding to the period 1996–2005. A ten-year averaging period removes the influence of the El Niño Southern Oscillation (return period ~5 years) on variability and highlights longer-term trends. Figure 1a displays a similar pattern for each measure of hurricane activity: during the 1950s and early 1960s Atlantic basin activity was 20–40% above the norm; during the 1970s and 1980s activity fell to 20–40% below the norm; by 1996–2005 it had risen substantially to reach 40–70% above the norm. For US-landfalling activity (Fig. 1b) the time series is more noisy but the general pattern of Fig. 1a is replicated, showing that tropical Atlantic activity and US-landfalling hurricane activity are positively linked when averages are taken over several years.

The contribution of sea warming to the recent exceptionally high hurricane frequency and activity is examined by using a statistical model with a sound physical basis. We apply the model from 1965 because North Atlantic hurricane records (and environmental fields) are considered most reliable from this time[3,25]. This model replicates a large proportion (75–80%) of the variance in tropical Atlantic hurricane activity between 1965 and 2005 from a knowledge of just two environmental fields: one, sea surface temperature; the other, an atmospheric wind field. These fields are the anomaly in August–September zonal (east–west) trade wind speed, u_T, at 925 hPa over the Caribbean and tropical North Atlantic (region 7.5–17.5° N, 30–100° W), and the anomaly in August–September SST over the

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tropical North Atlantic (region 10–20° N, 20–60° W; termed here the hurricane main development region (MDR) but comprising ~60% of the 10–20° N latitude belt termed the hurricane MDR in ref. 26). The wind field influences cyclonic vorticity and vertical wind shear over the main hurricane track region. Cyclonic vorticity either helps or hinders the spinning up of storms, depending on the sign and magnitude of its anomaly. Vertical wind shear either helps a vertically coherent storm vortex to develop or hinders it from doing so, depending on its magnitude. The oceanic thermal field provides heat and moisture to help power the development of storms within the MDR. August and September are the main months for North Atlantic hurricane activity, with 70% of the annual ACE index occurring in this period.

The central role of these two environmental fields in annual hurricane activity is shown in Fig. 2 and Table 1. Figure 2a displays the composite difference in SST and 925-hPa vector winds between subset years when the ACE index 1965–2005 is in its upper and lower quartiles; it thus shows the anomalies in SST and 925-hPa wind linked to active hurricane seasons during August and September. White rectangles mark the areas used for the two key environmental fields. Active hurricane seasons are associated with below-norm August–September 925-hPa trade winds and above-norm August–September SST in the MDR. Assuming perfect knowledge of these two fields we apply multiple linear regression[27–29] with cross-validation and block elimination[29,30] to compute hindcasts (forecasts run retrospectively) for the four different measures of tropical Atlantic hurricane activity during 1965–2005. The hindcast time series are compared with actual values for the ACE index and numbers of hurricanes in Fig. 2b and Fig. 2c, respectively. The model precision is impressive, explaining 81% and 76% of the variance in these two measures between 1965 and 2005. The key role of the two August–September environmental fields is further demonstrated by their anomaly time series (Fig. 2d, e), which display long-term trends similar to the trend in hurricane activity (Fig. 1). Furthermore, their 1996–2005 SST anomaly value of 0.27 °C is the highest ten-year anomaly since records began in 1950.

The hindcast correlation skill for tropical Atlantic hurricane frequency and activity during 1965–2005 is compared for four different statistical models in Table 1. These models comprise two with single predictors and two with two predictors. The single-predictor models employ the August–September SST in the MDR and the August–September 925-hPa u_T wind. The two-predictor models use the August–September SST in the MDR and the August–September 925–hPa u_T wind (as above and in the third row in Table 1), and the August–September SST in the MDR and the August–September 850–200-hPa vertical wind shear. The area employed for the latter is

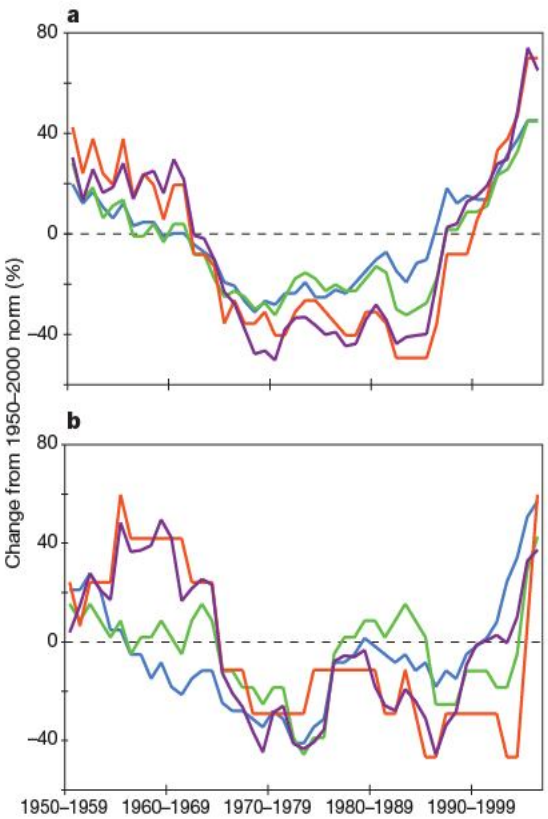

Figure 1 | The recent exceptionally high hurricane frequency and activity. Ten-year running averages for different measures of tropical Atlantic (**a**) and US-landfalling (**b**) hurricane frequency and activity between 1950 and 2005 are expressed as percentage departures from norm values for 1950–2000. The measures shown are numbers of tropical storms (blue), numbers of hurricanes (green), numbers of intense hurricanes (red) and the ACE index (purple)[18]. For **b** these measures refer to US-landfalling events, and ACE is the US ACE index[29].

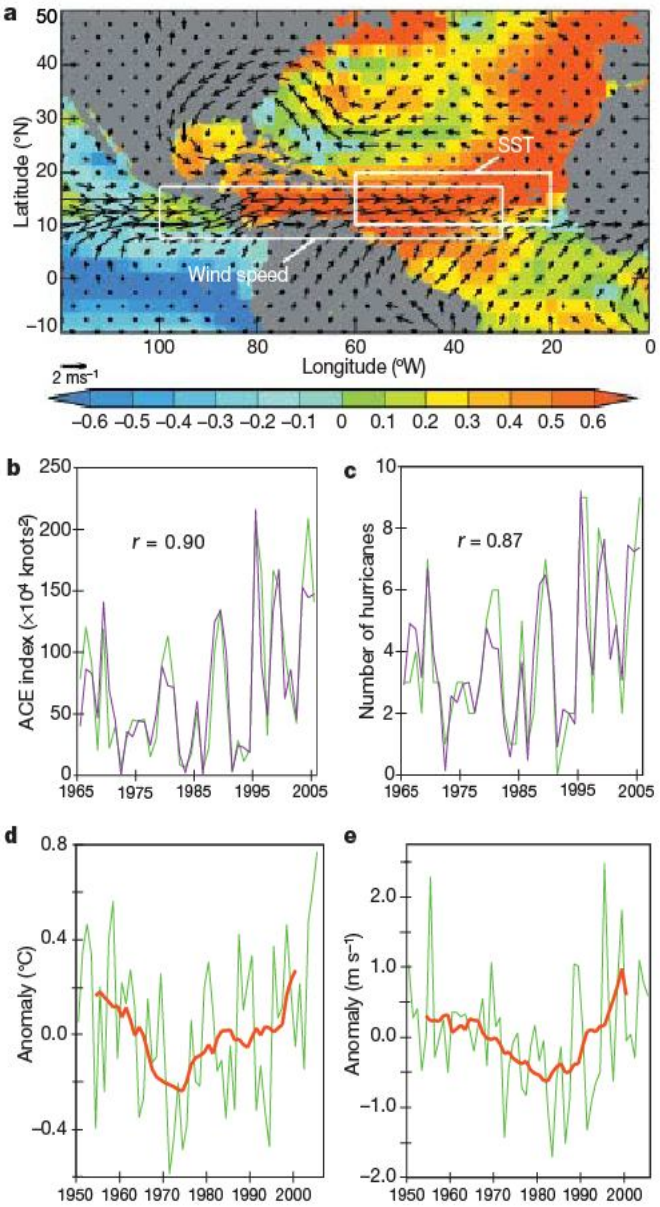

Figure 2 | Nature and performance of the statistical model replicating hurricane frequency and activity in the tropical Atlantic between 1965 and 2005. **a**, The two August–September environmental field areas that comprise the model and the August–September anomalies in SST (colour-coded in degrees Celsius) and 925-hPa wind anomalies (arrowed) linked to active Atlantic hurricane years. **b**, **c**, Comparison of the model's hindcast performance (purple) with actual values (green) for the ACE index (**b**) and number of hurricanes (**c**). **d**, **e**, The anomaly time series relative to the norm for 1950–2000 (green) and its associated ten-year running average (red) for the two environmental fields comprising the model, namely August–September SST (**d**) and 925-hPa u_T wind (**e**).

Table 1 | Predictive skill 1965–2005 as a function of predictor(s) and hurricane frequency/activity measure

Predictor(s)	Hindcast correlation skill			
	Numbers of tropical storms	Numbers of hurricanes	Numbers of intense hurricanes	ACE index
August–September MDR SST	0.68	0.68	0.64	0.71
August–September 925-hPa u_T wind	0.80	0.78	0.73	0.83
August–September MDR SST and August–September 925-hPa u_T wind	0.86	0.87	0.79	0.90
August–September MDR SST and August–September 200–850-hPa vertical wind shear	0.78	0.81	0.74	0.83

The MDR area is 10–20° N, 20–60° W. The 925-hPa u_T wind area is 7.5–17.5° N, 30–100° W. The region used for the 200–850-hPa vertical wind shear is 12.5–17.5° N, 40–85° W. Hindcast correlation skill is the Pearson product–moment correlation between the predicted and actual time series.

selected to maximize hindcast skill. Table 1 shows that the two-predictor model with the August–September SST in the MDR and the August–September 925-hPa u_T wind performs best for each measure of hurricane activity. Table 1 also shows that the August–September SST in the MDR alone explains 40–50% of the variance in hurricane activity during 1965–2005, and that the August–September 925-hPa u_T wind individually explains more variance than the SST does.

We also asked how sensitive the tropical Atlantic hurricane frequency and activity is to increasing August–September SST in the MDR and how much of the 40–70% above-norm elevated hurricane activity during 1996–2005 (Fig. 1) is linked to the 0.27 °C above-norm Atlantic August–September SST in the MDR during 1996–2005 (Fig. 2d). We addressed these questions by using our statistical model and removing the influence of atmospheric wind. Because the SST and wind factors together explain 75–80% of the variance in hurricane activity during 1965–2005, the differences in storm numbers and activity that remain after removal of the influence of wind are assumed to be due to the difference in SST. Removal of the influence of wind is achieved through multiple linear regression (see Methods Summary).

Figure 3 displays the deduced contribution from sea warming to the increase in hurricane frequency and activity between 1965 and 2005. Increasing the August–September SST in the MDR by 0.5 °C above its climate norm value of 27.3 °C is linked to increases above the 1950–2000 norm values of 31 ± 17% (numbers of tropical storms), 36 ± 17% (numbers of hurricanes), 45 ± 24% (numbers of intense hurricanes) and 49 ± 18% (ACE index), with uncertainties being the 95% confidence interval. The proportions of the above-norm storm frequency and activity during 1996–2005 (Fig. 1) linked to the 0.27 °C increase in the August–September SST in the MDR are as follows: 37 ± 20% (numbers of tropical storms), 46 ± 22% (numbers of hurricanes), 35 ± 19% (numbers of intense hurricanes) and 40 ± 15% (ACE index), with uncertainties again being the 95% confidence interval. The likelihood of a positive link between elevated August–September SST in the MDR and increased hurricane frequency and activity during 1996–2005 is more than 99% for all measures.

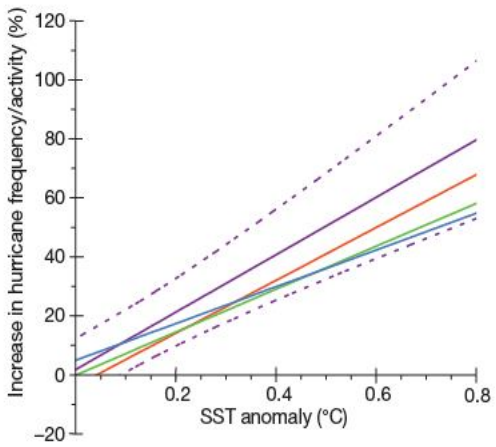

Figure 3 | Sensitivity of tropical Atlantic hurricane activity to increasing August–September SST in the MDR after removing the influence of atmospheric wind. The four frequency and activity measures are the same as in Fig. 1 (numbers of tropical storms (blue), numbers of hurricanes (green), numbers of intense hurricanes (red) and ACE index (purple). Linear regression fits for each measure and the 95% confidence interval for the ACE index fit (dashed purple lines) are shown. All fits are built on data from 1965–2005. The anomaly in SST in the MDR is relative to the 1950–2000 norm value of 27.31 °C. The percentage increases in hurricane frequency and activity are also relative to 1950–2000 norm values.

Our study shows that the current sensitivity of hurricane frequency to warming sea temperatures in the MDR is large: a 0.5 °C increase in August–September SST is linked to a ~40% increase in frequency. This finding pertains to storms forming in tropical regions of the North Atlantic from which 85–90% of US-landfalling hurricanes originate. The inclusion of tropical storms forming in the subtropics slightly reduces the sensitivity to a ~35% increase in frequency for a 0.5 °C increase in August–September SST in the MDR. In individual years the sensitivity will be higher or lower than this average. For example, during El Niño events the sensitivity will probably be lower.

METHODS SUMMARY

The influence of the August–September 925-hPa u_T wind on hurricane frequency and activity is removed by using a multiple linear regression[27–29] of the form $I = a + bS + cW$, where I is the hurricane frequency and activity measure in question, a is the intercept, b and c are constants, S is the August–September SST in the MDR and W is the August–September 925-hPa u_T wind speed. The value of b is the magnitude of I attributable to S with W held constant. The sensitivity to sea warming deduced with the multiple-regression approach is similar to that obtained with a simpler method. The latter selects all 'year-pairs' between 1965 and 2005 for which W differs by less than 0.1 m s^{-1} (a threshold equivalent to a change in storm numbers and activity of less than 5%). Because S and W together explain a large proportion (75–80%) of the variance in hurricane activity during 1965–2005, the difference in hurricane numbers and activity between these year-pairs is assumed to be attributable to the difference in S. Plotting these differences for every 'year-pair' allows the sensitivity to sea warming to be deduced.

The 95% confidence interval for the ACE index regression line in Fig. 3 is computed from equation 5.16 in ref. 28. This confidence band and similar ones (not shown) for the regression lines for tropical storms, hurricanes and intense hurricanes allow the computation of the 95% confidence intervals for how much of the elevated storm frequency and activity during 1996–2005 may be linked to the 0.27 °C increase in August–September SST in the MDR.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions M.A.S. instigated and directed the research and wrote the manuscript. A.S.L. performed the data analysis and contributed ideas.

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LETTERS

A great earthquake doublet and seismic stress transfer cycle in the central Kuril islands

Charles J. Ammon[1], Hiroo Kanamori[2] & Thorne Lay[3]

Temporal variations of the frictional resistance on subduction-zone plate boundary faults associated with the stick–slip cycle of large interplate earthquakes are thought to modulate the stress regime and earthquake activity within the subducting oceanic plate[1–3]. Here we report on two great earthquakes that occurred near the Kuril islands, which shed light on this process and demonstrate the enhanced seismic hazard accompanying triggered faulting. On 15 November 2006, an event of moment magnitude 8.3 ruptured the shallow-dipping plate boundary along which the Pacific plate descends beneath the central Kuril arc. The thrust ruptured a seismic gap that previously had uncertain seismogenic potential[4,5], although the earlier occurrence of outer-rise compressional events had suggested the presence of frictional resistance[1,2]. Within minutes of this large underthrusting event, intraplate extensional earthquakes commenced in the outer rise region seaward of the Kuril trench, and on 13 January 2007, an event of moment magnitude 8.1 ruptured a normal fault extending through the upper portion of the Pacific plate, producing one of the largest recorded shallow extensional earthquakes. This energetic earthquake sequence demonstrates the stress transfer process within the subducting lithosphere, and the distinct rupture characteristics of these great earthquakes illuminate differences in seismogenic properties and seismic hazard of such interplate and intraplate faults.

Earthquakes usually occur as sequences involving a few (or no) relatively small foreshocks, a mainshock, and a rapidly decaying number of aftershocks, with the mainshock typically being about a magnitude unit larger than the largest aftershock. Occasionally, a large earthquake is soon followed by an event of comparable size, either on an adjacent portion of the fault that ruptured initially or on a separate fault, and such events are termed doublets[6,7]. Earthquake doublets present particular challenges for seismic hazard assessment after a large event, but also provide insights into earthquake clustering, triggering and stress cycling[6–10].

The recent events in the central Kuril islands arc (Fig. 1) comprise one of the largest great earthquake doublets on record. In late September 2006, a swarm of thrust faulting foreshocks, including two of moderate magnitude (~6.6), ruptured the plate boundary east of the Kuril islands. Subsequently, the great thrust event occurred on 15 November 2006. The US Geological Survey (USGS) source parameters (http://earthquake.usgs.gov/regional/world/historical.php) for this earthquake are: 11:14:13.570 UTC, 46.592° N, 153.266° E, body-wave magnitude $m_b = 6.5$ and surface-wave magnitude $M_s = 7.8$. Thrust-faulting aftershocks (http://www.globalcmt.org/CMTsearch.html) of this event were distributed 250 km along the arc (Figs 1 and 2 and Supplementary Fig. 1). A parallel band of aftershock activity in the outer rise, eventually extending more than 200 km along the arc, initiated within minutes and continued for two months, the larger events having normal faulting mechanisms (http://www.globalcmt.org/CMTsearch.html). The 13 January 2007 great earthquake ruptured a normal fault in the outer rise roughly parallel to the rupture zone of the thrust event. The USGS source parameters (http://earthquake.usgs.gov/regional/world/historical.php) for this earthquake are: 04:23:21.160 UTC, 46.243° N, 154.524° E, $m_b = 7.3$ and $M_s = 8.2$. The aftershock sequence following the great outer rise event was less intense than that for the earlier thrust (Fig. 2), but also extended several hundred kilometres along the trench. The sequence resulted in an extraordinary double band of seismic activity (Fig. 1 and Supplementary Fig. 1).

The similarity in seismic moment of the two largest events classifies them as a doublet involving the rupture of distinct faults in close (~100 km) spatial proximity. Despite their large magnitudes, these events generated modest tsunamis only, less than a metre high in most locations for the November event, with the largest reported value being 1.76 m at Crescent City, California, at which location the January event produced a maximum tsunami height of only 0.37 m. In most locations the January event tsunami was less than half the size of that for the November event (http://earthquake.usgs.gov/regional/world/historical.php).

Seismic waves from the Kuril islands doublet were recorded by hundreds of global broadband seismographs, enabling detailed characterizations of their rupture processes. Finite-source inversions of azimuthally distributed teleseismic P and SH waveforms and Rayleigh-wave effective source time functions (STFs)[11–13] yielded slip distributions on faults with geometries constrained by Global Centroid-Moment Tensor (GCMT) solutions (http://www.globalcmt.org/CMTsearch.html). Our preferred model for the 15 November 2006 event has a fault orientation of strike 215° and dip 15°, with nearly pure thrust motion with a rake of 92° (Supplementary Fig. 2). The aftershock distribution favours this shallow-dipping interplate plane as the fault plane. A rupture velocity of 2.0 km s^{-1} is constrained by surface-wave directivity, and the rupture lasted about 120 s. Our seismic moment is 4.6×10^{21} N m, equivalent to a moment magnitude of $M_w = 8.4$. For the 13 January 2007 normal faulting event, our rupture model geometry has a strike of 43°, a dip of 59°, and a rake of −115° (Supplementary Fig. 3). This rupture's compactness makes it hard to distinguish between rupture on this plane versus rupture on the auxiliary plane (http://www.globalcmt.org/CMTsearch.html), but the southeast-dipping plane of the GCMT solution aligns better with the aftershock distribution. We do not exclude the possibility of rupture on a rotated northwest-dipping plane. The model's rupture velocity is 3.5 km s^{-1} and the main rupture lasted about 40 s, with some weaker radiation that is spatially poorly resolved lasting for another 20 s. Our seismic moment, 1.5×10^{21} N m ($M_w = 8.1$), places this as the third largest outer rise normal faulting event recorded, after the 1933 Sanriku,

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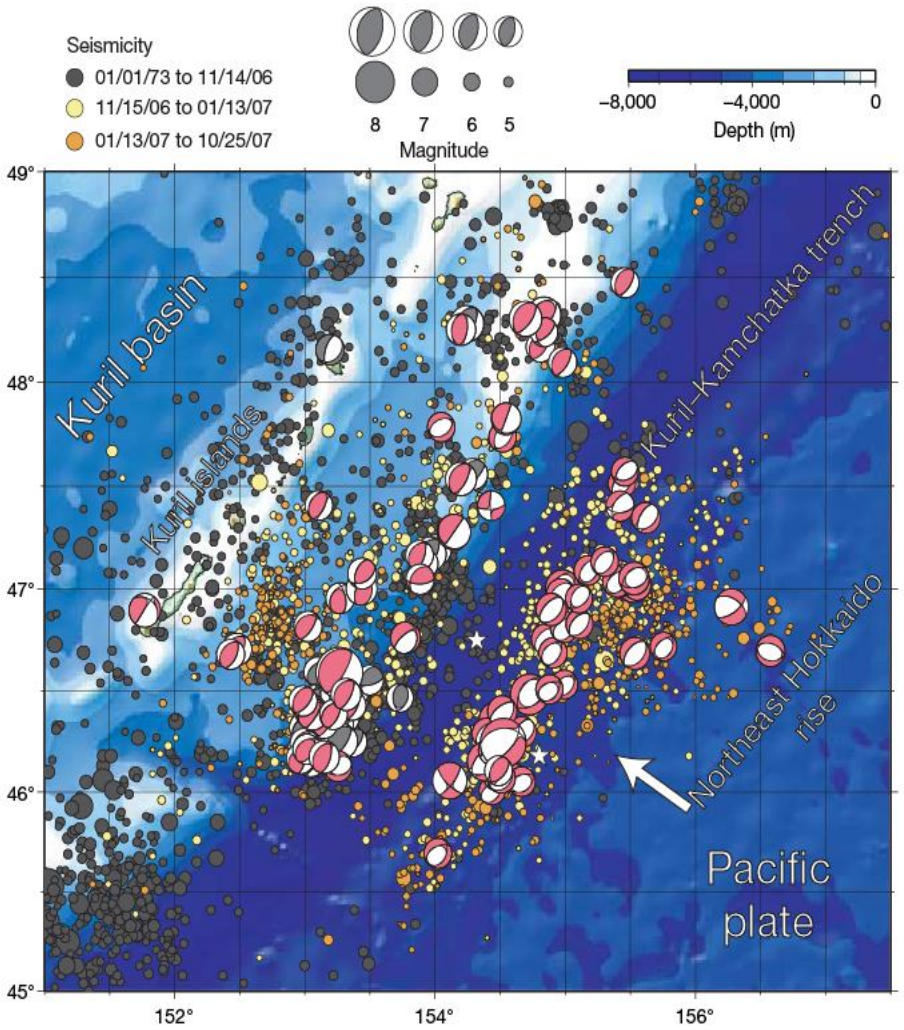

Figure 1 | Great doublet rupture region. Central Kuril islands earthquake locations (circles) from the USGS National Earthquake Information Center (NEIC) catalogue and lower hemisphere GCMT solutions (http://www.globalcmt.org/CMTsearch.html). Epicentres are colour-coded to show activity before 15 November 2006 (grey), between the doublet events (yellow), and after the 13 January 2007 event (orange). Focal mechanisms of foreshocks of the 15 November 2006 event are grey, subsequent events are red. Focal mechanisms are plotted at the NEIC epicentres; the stars are the GCMT centroid locations for the doublet events. The arrow indicates the direction of motion of the Pacific plate at about 80 mm yr^{-1}.

Japan ($M_w = 8.4$) and 1977 Sumba, Indonesia ($M_w = 8.3$) earthquakes. The slip distributions are shown in Fig. 3, and the very different moment rate functions are shown in Fig. 4.

Body-wave spectral amplitudes for the 13 January 2007 event are significantly larger, by ratios of 4 to 7, than those for the 15 November 2006 event, despite the larger seismic moment of the earlier event (Fig. 4). The January event thus has a larger 1-s-period m_b and a 20-s-period M_s than the November event. This short-period enrichment is similar to that for the 1933 Sanriku earthquake[14], and may reflect rupture on a fault with little cumulative slip. Seismic energy release for the November event (9.6×10^{15} J) is less than for the January event (4.3×10^{16} J), and the energy-moment ratios are 2.7×10^{-6} and 2.4×10^{-5}, respectively[15]. The factor-of-9 contrast in scaled energy indicates significant differences between interplate and intraplate faulting environments. Triggering of a large outer rise rupture with strong high-frequency shaking constitutes an important potential seismic hazard that needs to be considered in other regions.

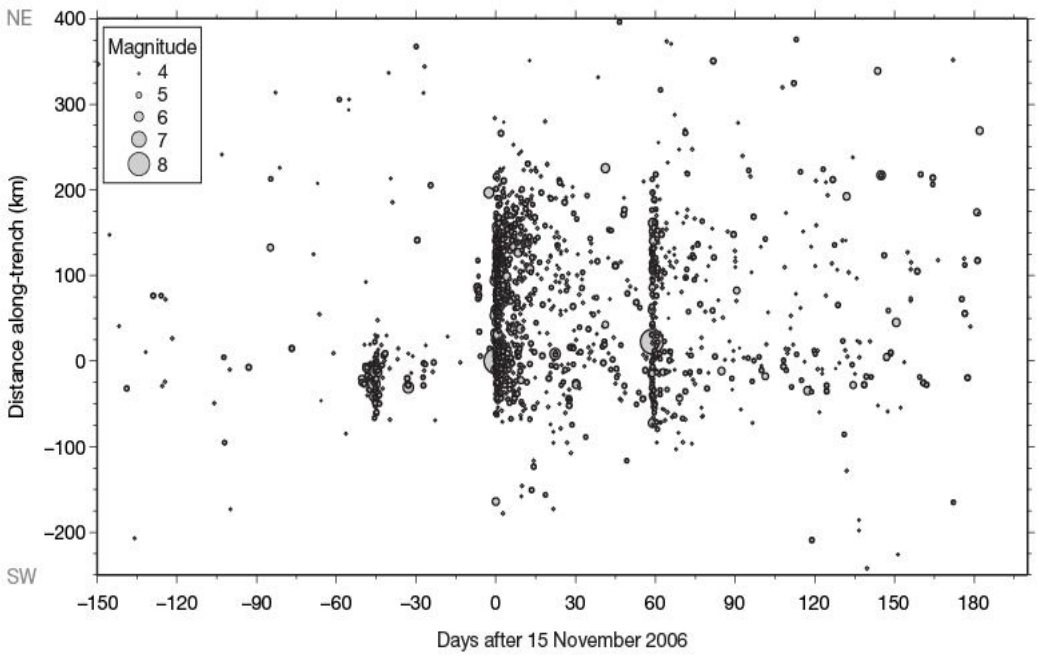

Figure 2 | Seismicity pattern. Space–time seismicity pattern for the 2006–2007 Kuril islands earthquake sequence, as a function of time relative to the 15 November 2006 event and a function of distance along the trench relative to that event's epicentre. The foreshock sequence 45 days before the November event (Fig. 1) and the two main-shock sequences are distinct in time, although many of the early aftershocks of the November event are located in the outer rise (Fig. 1) where the normal fault ruptured 60 days later.

This doublet is located in what had been a ~500-km-wide seismic gap northeast of the 1963 Kuril islands earthquake ($M_w = 8.5$) rupture zone and southwest of the 1952 Kamchatka earthquake ($M_w = 9.0$) rupture zone (Supplementary Figs 4 and 5). Only one large ($M_s \approx 8$) shallow event, in 1915, was located in this seismic gap in the last century, and the potential for great interplate earthquakes occurring in this region was unclear[4,5] before the 15 November 2006 event. Some evidence suggests that the former seismic gap region relative to the adjacent seismogenic regions along the arc has distinctive physical properties: relatively high trench-parallel gravity anomalies along the arc[16], a seaward offset in the shallow seismicity distribution along the arc, and narrowing of the trench (Fig. 1 and Supplementary Fig. 1). The age of the subducting oceanic lithosphere is probably over a hundred million years. The doublet occurred in the only region of the Kuril islands trench with a significant fore-arc basin, bordered on the south by a large sea-floor canyon (near 46° N, 152.5° E). Large seismic slip is located under forearc basins elsewhere[17], and the 15 November 2006 rupture supports the idea of upper plate influence on seismic coupling. The Pacific plate subducts at a rate of ~80 mm yr^{-1} beneath the arc in a direction of ~N60° W, generating great earthquakes with recurrence intervals of 100–200 yr elsewhere along the arc (Supplementary Fig. 5), but the relative proportion of seismic versus aseismic convergence in the central region has been debated.

Outer-rise normal faulting events are commonly attributed to bending of the oceanic lithosphere, which places the shallow portion of the slab in trench-perpendicular extension and the deeper part, below a neutral stress surface, in trench-perpendicular compression[1,18]. Normal fault offsets near the surface in the outer rise are typically less than ~100 m, so the faults are probably relatively fresh compared to the megathrust fault, which experiences many ruptures and huge total offsets[19]. The 2006–2007 extensional activity is primarily located along the trenchward edge of the outer rise. In a few regions, such as offshore of Honshu, Japan[14], and Sumba, Indonesia[20], great normal faulting events have occurred seaward of regions of weak seismic coupling, and this has been attributed to slab-pull stresses breaking or detaching the sinking slab by rupturing through the entire oceanic lithosphere[14].

Moderately sized shallow outer rise extensional faulting is usually observed after great thrust events[1,21,22], which indicates that stress perturbations associated with the cycle of interplate thrusting events affect the outer rise stress environment (Supplementary Fig. 6). Large outer rise compressional events are rare, but tend to precede large interplate thrusts[1]. The last few decades of outer rise activity along the Kuril islands arc primarily involved extensional faulting seaward of earlier large interplate thrusts, with the exception of several outer rise compressional events distributed along the former seismic gap (Supplementary Fig. 4). These include a large $M_s = 7.2$ event on 16 March 1963 (46.79° N, 154.83° N; ref. 1), located in the outer rise offshore of the 15 November 2006 rupture zone (Fig. 3). This compressional outer rise activity was invoked as one line of evidence favouring seismogenic potential and frictional locking of the seismic gap[1,2] before the 2006 event.

The transition from outer rise compression to outer rise tension following the 2006 interplate thrust supports the notion of outer rise stress modulation by varying interplate frictional stresses[22–24]. The Kuril doublet provides the clearest example of the full temporal pattern through the seismic cycle yet observed. The January outer rise event was unusually large, comparable to the great extensional events in uncoupled seismic zones, suggesting that the November event completely relaxed friction on the megathrust, allowing slab-pull forces to operate unimpeded on the outer rise. Stress transfer occurred on multiple timescales. Initial outer rise activity commenced within 40 min of the large thrust event, suggesting that

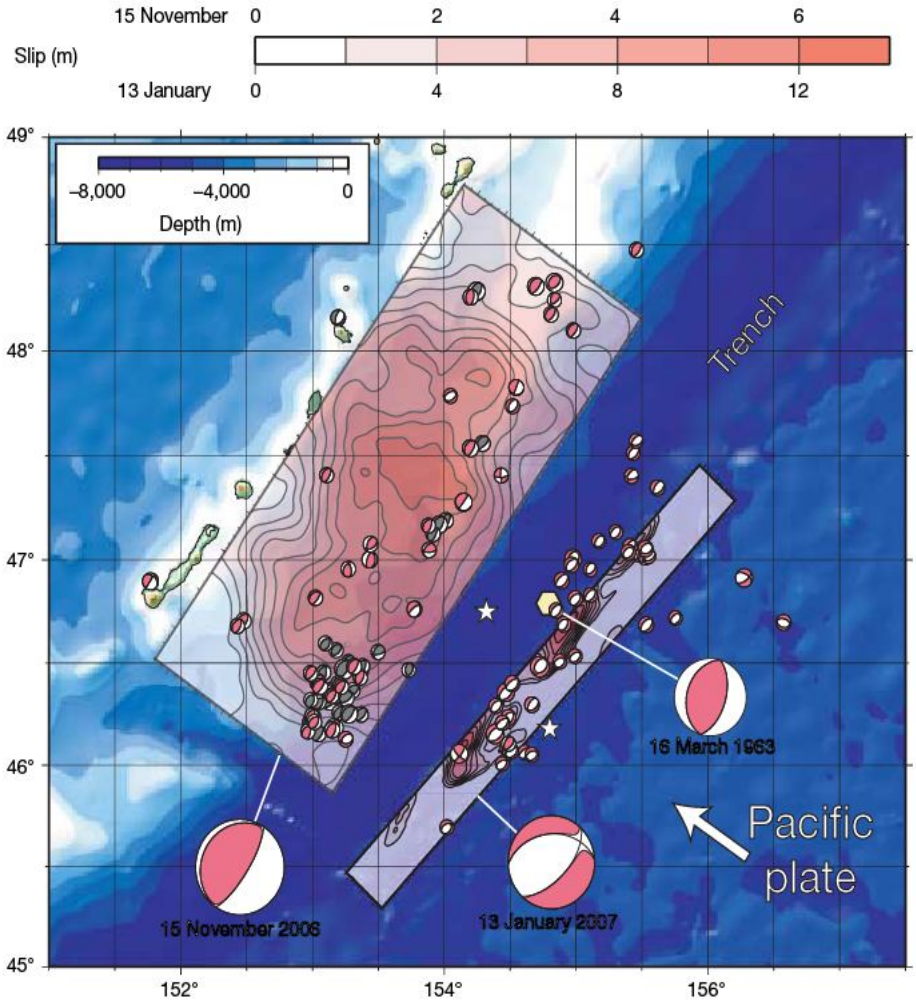

Figure 3 | Coseismic slip distributions. Surface projection of coseismic slip for the 15 November 2006 (average slip 4.6 m) and 13 January 2007 (average slip 9.6 m) events (NEIC epicentres shown by yellow circles, GCMT centroid epicentres shown by stars). GCMT mechanisms (centred on NEIC epicentres) for large events between June 2006 and May 2007 are shown; enlarged mechanisms are shown for the doublet events. Grey mechanisms indicate events before the 15 November 2006 event, red mechanisms indicate events after that rupture. The focal mechanism and epicentre of the 16 March 1963 compressional outer-rise event (yellow hexagon) are included. The arrow indicates the direction of the Pacific plate motion at 80 mm yr^{-1}.

dynamic or static stress transfer triggered events in a highly strained segment of the Pacific plate. The 60-day delay before the second member of the doublet indicates a longer response time, consistent with a visco-elastic strain migration rate of 100 km per 60 days, comparable to that for the $M_w = 7.7$ outer rise earthquake of 30 March 1965, triggered by the great 4 February 1965 Rat Island earthquake[25].

Outer-rise normal faulting probably plays a critical role in hydrating the downgoing oceanic lithosphere[26]. It is significant that the 13 January 2007 rupture extended deeply into the upper mantle of the Pacific plate. Hydrothermal activity near mid-ocean ridges appears to be capable of hydrating the upper oceanic crust, but not the deep crust and upper mantle[26]. For faults that form in the outer rise, hydration of the shallow crust is likely to be localized near the fault structures along which water percolates or is dynamically pumped to depth, but sepentinization may broaden into a wider zone within the upper mantle[27]. Most evidence for upper mantle metamorphism of oceanic lithosphere is indirect, such as the suggestion that dewatering of metamorphosed serpentinite can explain intermediate-depth (50–200 km) earthquakes in the sinking lithosphere[26]. Some reflection–refraction data suggest unusually slow seismic wave speeds within the crust and uppermost mantle in outer-rise regions[27], favouring hydration along faults that penetrate deeply into the lithosphere. The scars from this doublet may host many future earthquakes.

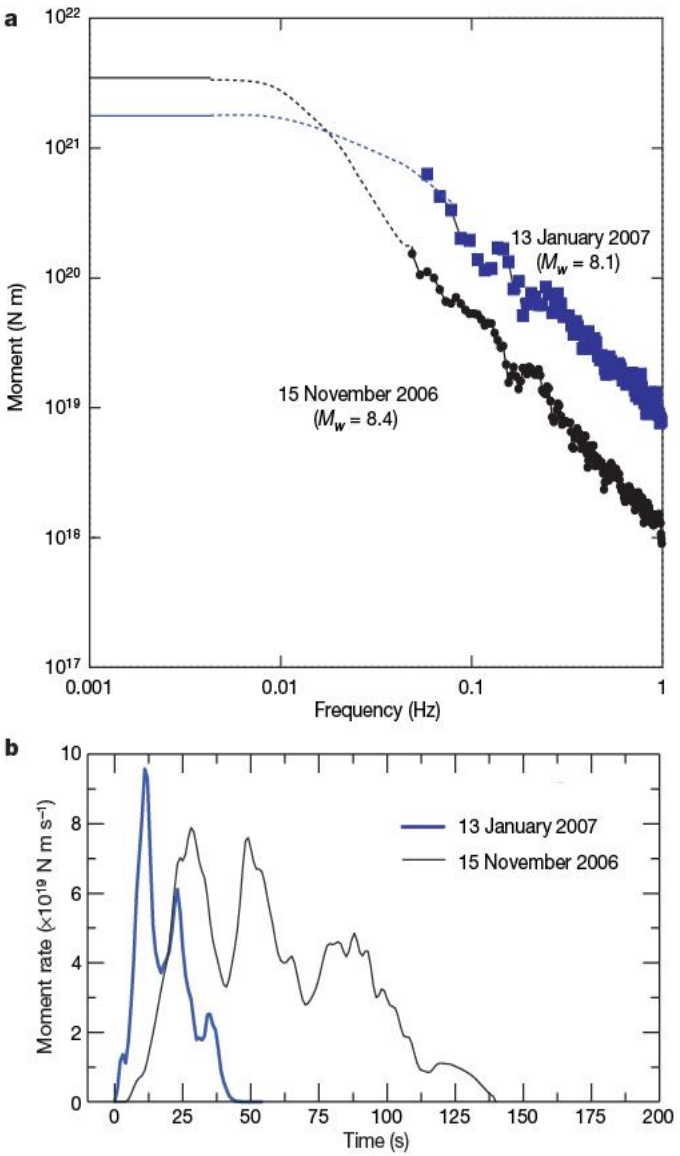

Figure 4 | Source radiation characteristics. **a**, Moment rate spectra for the 15 November 2006 and 13 January 2007 events. Note the larger high-frequency amplitudes for the smaller January event. This is associated with higher energy release and higher energy-to-seismic-moment ratio for the January event. **b**, STFs for the doublet events. We note the differences in total duration and overall complexity.

METHODS SUMMARY

The rupture models for the two great earthquakes are inverted from broadband seismic P and SH body-wave and R1 surface-wave signals spanning the period from 2 to ~750 s. The R1 signals were pre-processed by a deconvolution procedure to remove dispersion and frequency-dependent excitation effects for a standard Earth model and specified focal mechanism and source depth[13], yielding effective R1 STFs. A search-based algorithm[12] produces rupture models with a smooth, least-squares seismic moment distribution matching the observed signals. Details of the rupture are controlled by the P and SH waveforms. The primary contributions of the R1 STFs to the inversion are constraints on the smoother features, including the overall rupture directivity and seismic moment.

In the local-search algorithm, the rupture model is successively perturbed in a search for better-fitting models. Each search began with a zero-slip initial model and proceeded for several thousand perturbations, adopting the perturbed model each time an improvement in fit was found. The inversion is parameterized in terms of point-source strength, which converts to slip by assuming that each point source represents a sub-event with dimensions equal to the distance between sources (10 km for the 15 November 2006 event and 5 km for the 13 January event 2007) and shear moduli of $\mu = 40$ GPa (November event) and $\mu = 52$ GPa (January event).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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LETTERS

The adaptive significance of temperature-dependent sex determination in a reptile

D. A. Warner[1]† & R. Shine[1]

Understanding the mechanisms that determine an individual's sex remains a primary challenge for evolutionary biology. Chromosome-based systems (genotypic sex determination) that generate roughly equal numbers of sons and daughters accord with theory[1], but the adaptive significance of environmental sex determination (that is, when embryonic environmental conditions determine offspring sex, ESD) is a major unsolved problem[2,3]. Theoretical models predict that selection should favour ESD over genotypic sex determination when the developmental environment differentially influences male versus female fitness (that is, the Charnov–Bull model)[4], but empirical evidence for this hypothesis remains elusive in amniote vertebrates—the clade in which ESD is most prevalent[5]. Here we provide the first substantial empirical support for this model by showing that incubation temperatures influence reproductive success of males differently than that of females in a short-lived lizard (*Amphibolurus muricatus*, Agamidae) with temperature-dependent sex determination. We incubated eggs at a variety of temperatures, and de-confounded sex and incubation temperature by using hormonal manipulations to embryos. We then raised lizards in field enclosures and quantified their lifetime reproductive success. Incubation temperature affected reproductive success differently in males versus females in exactly the way predicted by theory: the fitness of each sex was maximized by the incubation temperature that produces that sex. Our results provide unequivocal empirical support for the Charnov–Bull model for the adaptive significance of temperature-dependent sex determination in amniote vertebrates.

Why is an individual's sex determined by environmental variables (environmental sex determination, ESD) in some species, but by chromosomal factors (genotypic sex determination; GSD) in others? GSD plausibly enhances parental fitness by generating equal investment into sons versus daughters[1], but the adaptive significance of ESD remains a major unresolved problem, particularly for amniote vertebrates[2,3]. The problem is not a lack of plausible hypotheses, but rather the difficulty of testing those ideas. Mathematical models predict that ESD will be favoured by selection when an environmental variable (for example, temperature or photoperiod) differentially affects the fitness of sons versus daughters[4,6]. For example, the most common form of ESD is temperature-dependent sex determination (TSD), whereby incubation temperature determines offspring sex in many reptiles[5] and some fish[7]. The widely accepted Charnov–Bull model[4] predicts that TSD enhances parental fitness by matching offspring sex to incubation conditions; that is, eggs should produce sons when developing under conditions that promote high fitness for males, whereas eggs that encounter female-favourable conditions develop as daughters (see Fig. 1 for more detailed predictions). Genes creating such a link should be favoured by selection as they would confer higher fitness than the alternative GSD system.

Although the hypothesis and predictions are straightforward, tests of adaptive models are logistically difficult[8–12]. This is because, first, to evaluate sex-specific effects of incubation temperature, both sexes need to be produced at all incubation temperatures—an obvious problem if temperature determines offspring sex; second, most species with TSD exhibit long lifespans (60+ years) and delayed sexual maturation, precluding measurements of lifetime fitness; and, third, incubation temperature may differentially affect male versus female fitness by means of multiple pathways[3], complicating empirical tests of the Charnov–Bull model.

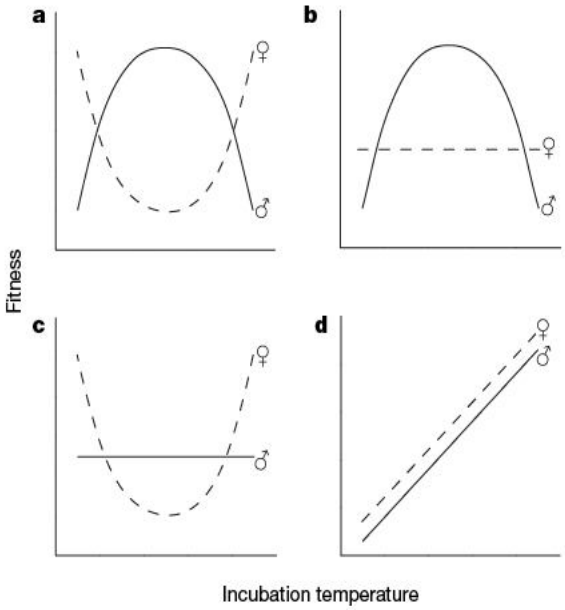

Figure 1 | Theoretical predictions of the Charnov–Bull model for the adaptive significance of TSD based on the TSD pattern in Jacky dragons (*A. muricatus*). In this species, females are produced at thermal extremes and males are produced at intermediate temperatures. The solid line represents fitness of sons and the dashed line represents fitness of daughters. The Charnov–Bull model predicts that TSD will enhance individual fitness if the fitness of sons is greatest for individuals that hatch from eggs incubated at temperatures that naturally produce males, and fitness of daughters is greatest for individuals from eggs incubated at temperatures that naturally produce females. These conditions might be satisfied if: **a**, male-producing temperatures are optimal for sons, and female-producing temperatures are optimal for daughters; **b**, female fitness is unaffected by incubation temperature, but fitness is optimized by intermediate incubation temperatures for males; or **c**, male fitness is unaffected by incubation temperature, but female fitness is optimized by cool and warm incubation temperatures. **d**, Many other scenarios should not favour TSD. For example, when incubation temperature affects fitness, but does so in similar directions for males versus females, TSD is not favoured by selection.

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We have overcome these obstacles in three ways: by hormonally manipulating eggs to decouple the confounded effects of sex and incubation temperature[13,14], by studying a short-lived species with TSD, and by using paternity analyses to evaluate the effects of incubation history on lifetime reproductive success for males as well as females. Our study organism, the Jacky dragon (*A. muricatus*), is a short-lived (probably 3–4 years) Australian agamid lizard with TSD[15]. Female offspring are produced from eggs incubated at low (23–26 °C) and high (30–33 °C) temperatures, and both sexes are produced at intermediate (27–30 °C) incubation temperatures[15]. We incubated eggs under each of these thermal regimes, and applied an aromatase inhibitor to half of the eggs early in development to override thermal effects on sex determination[16,17]. This manipulation blocked the conversion of testosterone to oestradiol during development, enabling us to produce male offspring at female-producing incubation temperatures. Thus, we were able to decouple the confounded effects of sex and incubation temperature. Importantly, this hormonal manipulation had no effect on morphology or survival of hatchling Jacky dragons[17], and gonadal histology showed that sex-reversed males do not differ from natural males[18]; similar non-effects of hormonal manipulations have been demonstrated in other reptiles[19,20]. Moreover, comparisons of males produced from eggs treated with an aromatase inhibitor versus naturally produced males (from 27 °C incubation) revealed no effect of aromatase inhibition on reproductive success ($F_{1,44} = 1.9$, $P = 0.17$). After eggs hatched, we followed the newly hatched individuals throughout their lives under semi-natural conditions by maintaining the lizards in large field enclosures for the next 3.5 years.

Incubation temperature had little direct effect on offspring phenotypes and survival, but had major effects through its covariation with the seasonal timing of hatching[17]. Warm incubation temperatures (that naturally produce daughters) accelerated embryonic development, allowing eggs to hatch early in the season. Consequently, incubation temperature had a strong positive effect (via its effect on the timing of hatching) on lizard body sizes before the onset of all three reproductive seasons monitored during this study (that is, when lizards were one, two and three years old; all P values <0.05). During the first reproductive season (2004), only two of the females produced viable offspring (two clutches comprising eight eggs in total); both females were from the warm (naturally female-producing) incubation treatment. The two males that sired their clutches were both from the intermediate (naturally male-producing) incubation treatment. These patterns fit the predictions of the Charnov–Bull model, albeit with small sample sizes.

At the onset of the second reproductive season (spring 2005), the minimum size at sexual maturity (72 mm snout-vent length[15]) had been attained by 70.6% and 69.0% of individuals from the intermediate and warm incubation treatments, respectively, but by only 37.2% of the individuals from the cool treatment ($\chi^2 = 11.1$, $P = 0.004$). Sixteen viable clutches (comprising 58 eggs in total) were produced during the second season, and our parentage analyses indicated that larger individuals (and hence, those that hatched early) were more likely to produce offspring (for both males and females, Fig. 2a; $F_{1,97} = 11.3$, $P = 0.001$). The same patterns (at least up to one year) occur under natural conditions in the field[21]. Reproductive success was strongly affected by the interaction between incubation temperature and sex (Fig. 3a, b; $F_{2,96} = 4.0$, $P = 0.021$) in a pattern consistent with the prediction in Fig. 1a. Similar to the patterns in the first year, males from eggs incubated at intermediate temperatures had the highest reproductive success, whereas more extreme (low and high) incubation temperatures enhanced female reproductive output.

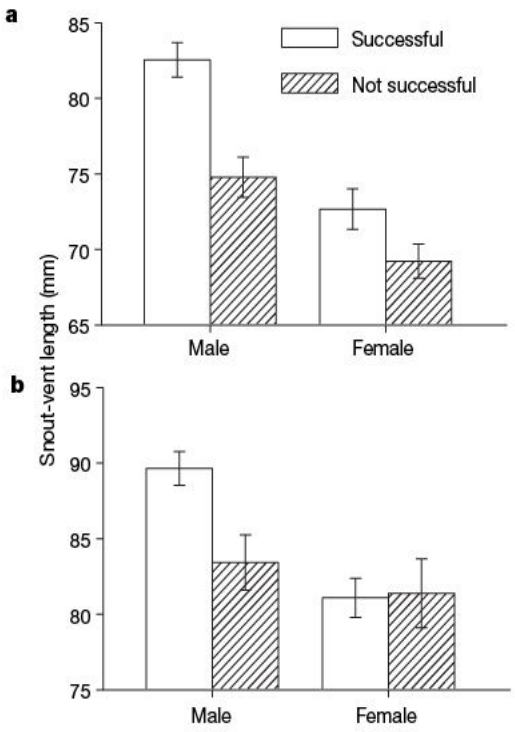

Figure 2 | Sex-specific body-size comparisons between individuals that successfully reproduced versus those that did not reproduce. Reproductive success was not evaluated statistically during the first reproductive season (2004–2005) because only two clutches were produced. **a**, Body-size comparisons in the second reproductive season (2005–2006). **b**, Body-size comparisons in the third reproductive season (2006–2007). Bars represent means ± 1 standard error.

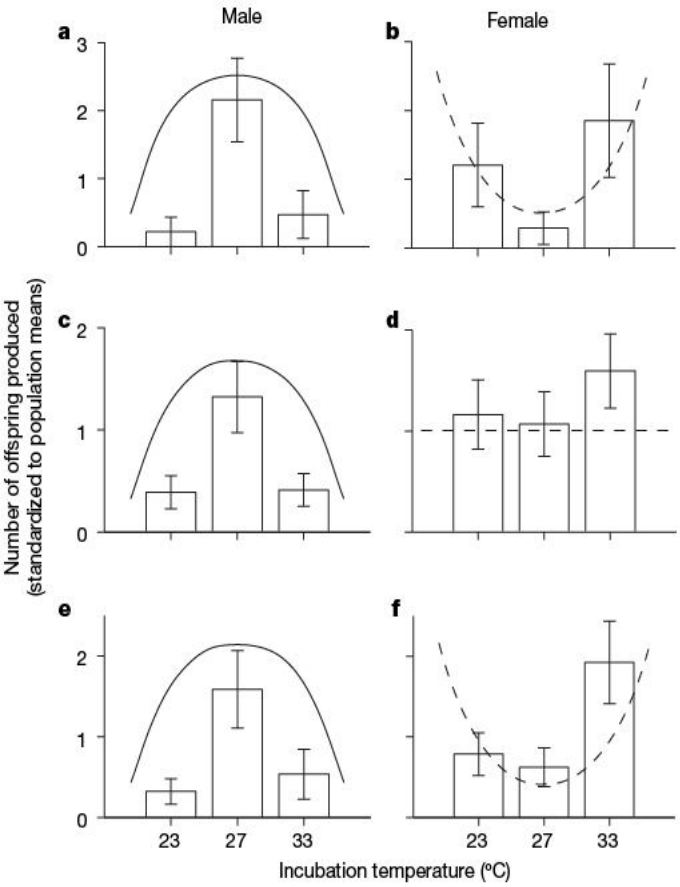

Figure 3 | Incubation temperature affected the fitness of sons differently from that of daughters. The left-hand panels show male reproductive success, and the right-hand panels show female reproductive success. Because only two clutches were produced in the first reproductive season, we did not include data from this season. **a–f**, The graphs show male (**a**) and female (**b**) reproductive success during the 2005–2006 season; male (**c**) and female (**d**) reproductive success during the 2006–2007 season; and male (**e**) and female (**f**) lifetime reproductive success, representing pooled reproductive data over all three seasons. Bars represent means ± 1 standard error. The curves on each graph represent the patterns predicted from Fig. 1 that best fit the results.

At the onset of the third reproductive season (spring 2006), all but three individuals (3.7%) had reached sexual maturity. Nonetheless, warmer-incubated (and thus, earlier-hatched) individuals still exhibited larger body sizes at this time (3 years after hatching; $F_{2,68} = 4.5$, $P = 0.015$). During this season, 50 clutches were produced (comprising 227 eggs). Larger body size again enhanced reproductive success for males, but not for females (Fig. 2b; interactive effect: $F_{1,71} = 4.3$, $P = 0.043$). Consequently, reproductive success followed a pattern broadly consistent with Charnov–Bull predictions, but different from that found in the second reproductive season (resembling Fig. 1b): male reproductive success was greatest for individuals produced at the intermediate incubation temperature, whereas female reproductive success was not significantly influenced by incubation treatment.

Lifetime reproductive success (pooled reproductive output for all three seasons, Fig. 3e, f) strongly conformed to the prediction in Fig. 1a (sex by temperature interaction: $F_{2,130} = 4.3$, $P = 0.016$). Males hatched from eggs incubated at naturally male-producing (intermediate) temperatures sired more offspring than did males from eggs incubated at naturally female-producing (extreme) temperatures. The reverse was true for females, with reproductive success greatest for females that hatched from eggs incubated at female-producing (low or high) temperatures. Thus, reproductive success of each sex was optimized by the incubation temperature that produces that sex in nature, as predicted by the differential-fitness (Charnov–Bull) model for the adaptive significance of TSD. These results provide the first unequivocal demonstration that incubation temperature differentially affects male versus female fitness in a way that will favour the evolution and maintenance of TSD in amniote vertebrates.

METHODS SUMMARY

Methodological details for the experimental design and early stages of the study are given elsewhere[17]. In spring 2003, we allocated eggs among three temperature regimes (23 °C, 27 °C or 33 °C) that mimicked natural nests[22]. Half the eggs in each treatment were given an aromatase inhibitor to produce male offspring at all temperatures[16]. After eggs hatched, all offspring were marked and raised in six replicate field enclosures for 3.5 years. Our releases ensured that each treatment and clutch were equally represented among replicate populations. When lizards became sexually mature, second-generation eggs were incubated, and tissue samples were taken from the resultant hatchlings. Samples were genotyped at nine microsatellite loci[23,24]. Paternity analyses were performed using CERVUS software (version 3.0.3)[25].

The effect of incubation temperature on body size at each season was evaluated with a mixed model analysis of variance (ANOVA) using temperature and sex as independent variables and enclosure as a random effect. Reproductive success was calculated as the number of offspring produced or sired by each individual that survived to the onset of each season; the number of offspring produced by each individual was standardized to the population mean for each enclosure. Two-way mixed model ANOVA was used to evaluate the interactive effect of sex and incubation temperature on reproductive success; enclosure was included as a random effect. Individuals that died throughout the study were not included in the within-season analyses, but were considered in the final analysis with pooled data across years (that is, lifetime reproductive success). The effect of body size on reproductive success was evaluated with mixed model ANOVA by comparing body sizes of individuals that reproduced versus those that did not. Before analysis, body size was standardized (as *z*-scores) for each enclosure, and enclosure was included as a random effect.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Contributions D.A.W. conducted the experiment, maintained the lizard populations, genotyped all individuals, analysed the data and wrote the first draft of the manuscript. Both authors contributed equally to the design of the experiment, discussion of the results and preparation of the final manuscript.

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LETTERS

Lethargus is a *Caenorhabditis elegans* sleep-like state

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There are fundamental similarities between sleep in mammals and quiescence in the arthropod *Drosophila melanogaster*, suggesting that sleep-like states are evolutionarily ancient[1–3]. The nematode *Caenorhabditis elegans* also has a quiescent behavioural state during a period called lethargus, which occurs before each of the four moults[4]. Like sleep, lethargus maintains a constant temporal relationship with the expression of the *C. elegans* Period homologue LIN-42 (ref. 5). Here we show that quiescence associated with lethargus has the additional sleep-like properties of reversibility, reduced responsiveness and homeostasis. We identify the cGMP-dependent protein kinase (PKG) gene *egl-4* as a regulator of sleep-like behaviour, and show that *egl-4* functions in sensory neurons to promote the *C. elegans* sleep-like state. Conserved effects on sleep-like behaviour of homologous genes in *C. elegans* and *Drosophila* suggest a common genetic regulation of sleep-like states in arthropods and nematodes. Our results indicate that *C. elegans* is a suitable model system for the study of sleep regulation. The association of this *C. elegans* sleep-like state with developmental changes that occur with larval moults suggests that sleep may have evolved to allow for developmental changes.

Behavioural quiescence is concentrated during lethargus—a period at larval-stage transitions (Fig. 1 and Supplementary Table 1). Each lethargus can be characterized by total quiescence, by the peak frequency of quiescent epochs in a 10-min period, and by the mean quiescence bout duration (Supplementary Fig. 2 and Supplementary Table 1). There is a rhythm to this process, with distinct lethargus periods that are consistent across animals (Fig. 1b and Supplementary Table 1).

A key feature of sleep is reduced sensory responsiveness. To determine if arousal threshold is increased during *C. elegans* lethargus, we tested responses to mechanical and olfactory stimuli, which are sensed by distinct neurons[6–8] (Fig. 2a).

The predominant response to dish-tap—a mechanical stimulus[9]—was a brief backward movement, both during and outside lethargus (Fig. 2b and Supplementary Video 3). Outside lethargus, the worm also frequently responded with complex behaviours (Fig. 2b). Therefore, lethargus represents a period of reduced responsiveness to mechanical stimulation. The fact that the worm always showed a response to this stimulation indicates that the mechanosensory circuit can function during lethargus.

We subjected the animal's nose to the chemical 1-octanol, which produces a withdrawal response. The response latency to diluted 1-octanol was increased during lethargus (Fig. 2c), yet animals remained responsive (Fig. 2d). After strong mechanical stimulation of the worms during lethargus, the response latency to 1-octanol was as short as during the fourth larval stage before lethargus (Fig. 3f). Therefore, the ASH sensory neurons can function normally during lethargus, and the reduced responsiveness is probably due to altered processing of sensory information.

Behavioural quiescence observed during lethargus is a reversible behavioural state. During lethargus, quiescent periods are interrupted by brief movements in which the animal assumes a sinusoidal posture—a posture assumed during normal locomotion (Supplementary Videos 1 and 2). Furthermore, response latency to 1-octanol is reduced to levels seen outside lethargus after strong mechanical stimulation of the animals (Fig. 3f). Finally, in response to strong mechanical stimulation, forward movement is as fast during lethargus as outside lethargus (Fig. 2e).

The homeostatic property of sleep[10] is manifested when, after a period of enforced wakefulness, subsequent sleep occurs with a reduced latency and is deeper, where depth of sleep is reflected by increased consolidation and reduced responsiveness[3,11]. To test for homeostasis, we stimulated worms mechanically beginning at nine hours after the end of the L3 lethargus—a time when most animals would display quiescent behaviour (Supplementary Table 1). On the basis of the behaviour of the unperturbed control group, our stimulation protocol is predicted to deprive the animals of 31% of the total quiescence in L4 lethargus. After the one-hour stimulation, the peak quiescence and the mean quiescence bout duration—two measures of consolidation—are increased (Fig. 3b, c). The timing of the end of the quiescent period is unaltered by the stimulation (Fig. 3d), indicating a probable temporal constraint on the timing of lethargus.

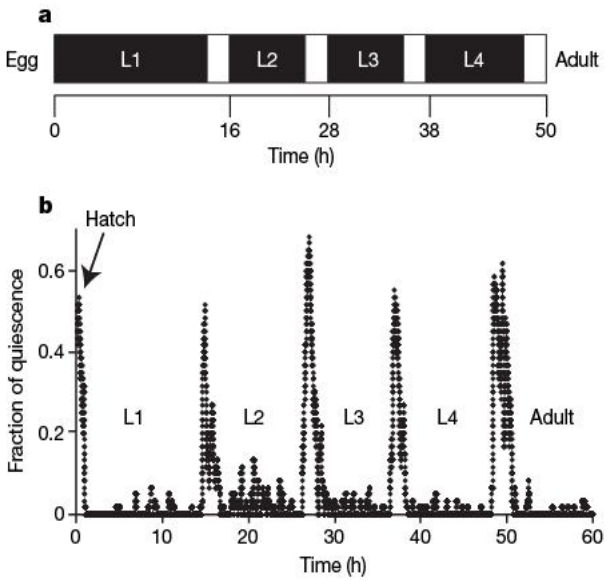

Figure 1 | Behavioural quiescence is concentrated during the lethargus periods. L1–L4 corresponds to larval stage 1 to larval stage 4. **a**, Postembryonic development of *C. elegans* at 20 °C. Lethargus is designated by the white rectangles. **b**, Shown is the fraction of quiescence of a single wild-type worm in a 10-min time window that is moved 10 s for each data point.

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This constraint would explain the overall reduction in total quiescence during L4 lethargus in deprived animals (Fig. 3a).

To assess for homeostasis further, we used the 1-octanol response latency to measure the time course of recovery to a sleep-like state. Animals that were deprived of quiescence and were kept continuously moving for 30 min during lethargus had, subsequently, an earlier occurrence of the long 1-octanol response latencies typical of lethargus in comparison to animals allowed to go through lethargus unperturbed (Fig. 3f). In addition, these animals showed an earlier cessation of locomotion in comparison to non-deprived animals (Fig. 3e). Finally, these deprived animals showed 1-octanol response latencies that were further increased compared to the latencies observed during lethargus (Fig. 3f), demonstrating a deeper sleep-like behaviour. Animals that were deprived of quiescence for 20 min during lethargus showed a latency to sleep-like behaviour that was intermediate to that seen after 30 min deprivation (Fig. 3e, f), indicating that this latency depends on the duration of previous deprivation as predicted for a sleep homeostatic process. Thirty minutes of activity during the early adult stage had no effect on subsequent 1-octanol response latencies and had a minimal effect on locomotion (Fig. 3e, f). We conclude that the sleep-like behaviour during lethargus is under homeostatic regulation.

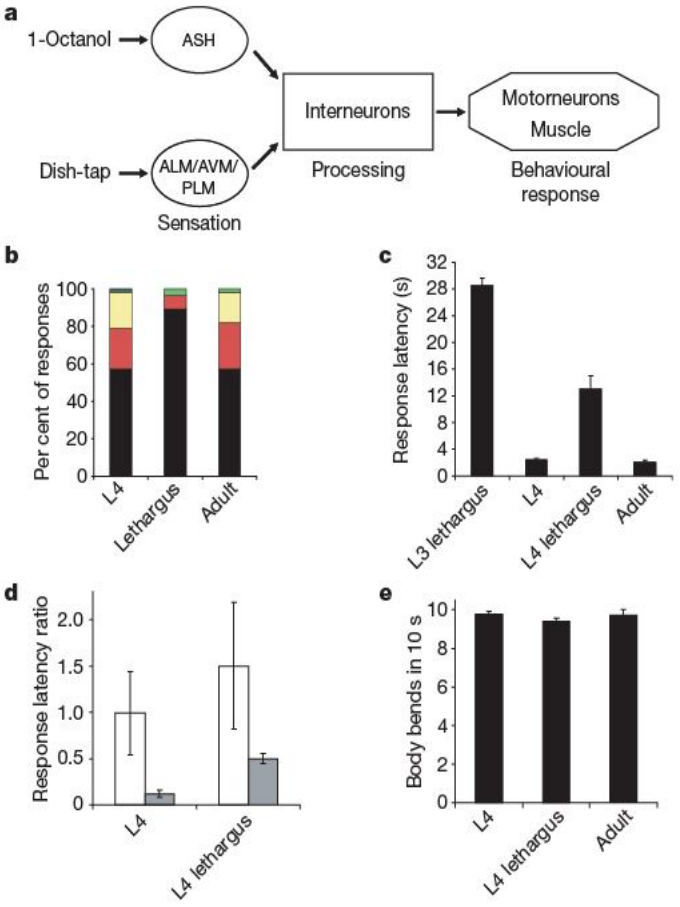

Figure 2 | Responsiveness is reduced during lethargus. **a**, Dish-tap is sensed by the mechanosensory neurons ALM, PLM and AVM[6], and dilute 1-octanol is sensed by the polymodal sensory neuron ASH[7]. **b**, In response to dish-tap, five behavioural responses were observed: brief backing (black); sustained backing (red); complex reorienting response (yellow); acceleration (green); and shrinking (blue). Shrinking was observed only once. The difference in the frequency distribution in the five categories between lethargus and the other two stages was significant at $P < 0.0001$ (chi-squared test). See Supplementary Information for additional details. **c**, Response latency to 30% 1-octanol is increased during lethargus. The mean ± s.e.m. response latency is shown for the L3 lethargus ($n = 20$), the L4 stage before lethargus ($n = 109$), the L4 lethargus ($n = 34$) and the adult stage ($n = 48$). Differences in response latency between the L3 lethargus and L4 stages, the L4 lethargus and L4 stages, and the L4 lethargus and adult stages were all significant at $P < 0.0001$, two-tailed Student's t-test with unequal variance. **d**, Worms respond to 30% 1-octanol during lethargus. Shown is the mean ± s.e.m. ratio of response latencies to two stimulations. The first stimulation consisted of 100% ethanol, and the second stimulation consisted either of 30% 1-octanol (grey) or of 100% ethanol (white). 'L4' and 'L4 lethargus' denote the fourth larval stage before and during lethargus, respectively. The effect of 1-octanol in comparison to that of ethanol was significant during both the L4 and the L4 lethargus stages at $P < 0.00001$ and $P = 0.01$, respectively (two-tailed Student's t-test, unequal variances). **e**, Continuous 10-s stimulation of the worms' tail during lethargus results in normal movement, as assessed by the number of anterior body bends. $n = 45$, 45 and 16 for the L4, L4 lethargus and adult stages, respectively. There was no difference between the speed during the L4 stage before lethargus and L4 lethargus ($P = 0.10$) or between adults and L4 lethargus ($P = 0.28$), two-tailed Student's t-tests. Shown is the mean ± s.e.m.

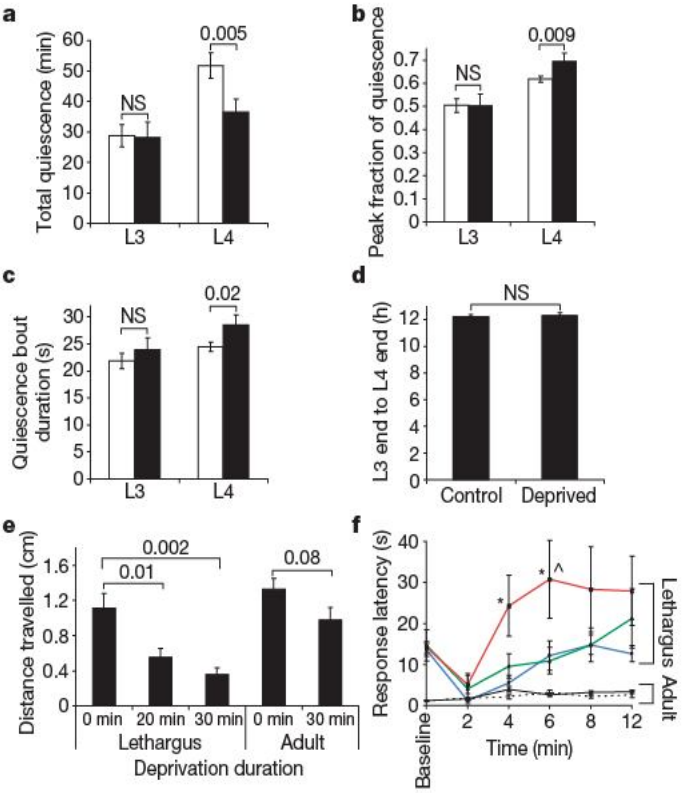

Figure 3 | Homeostatic regulation of lethargus. **a–d**, Mechanical stimulation for one hour beginning nine hours after the end of the L3 lethargus period results in reduced total quiescence (**a**) and increased quiescence consolidation (**b**, **c**) in the L4 lethargus period, but no change in the timing of the end of L4 lethargus (**d**). Shown are the mean ± s.e.m values from analysis of 18 unperturbed animals that began L4 lethargus at least nine hours after the end of L3 lethargus (white), and of 13 animals that were deprived of quiescence for one hour beginning nine hours after the end of the L3 lethargus period (black). NS denotes $P > 0.1$. Statistical significance was assessed with ANCOVA in **a–c** and with Student's t-test in **d**. **e**, The distance travelled by the worm in two minutes is reduced after deprivation of quiescence during L4 lethargus. P values are based on two-tailed Student's t-tests. $n = 10$ for each group. **f**, Prolonged 1-octanol response latencies observed during lethargus are reversible by strong stimulation, and recur with a faster time course and with further prolongation of the response latency after previous deprivation of quiescence. The x and y axes denote the time after strong stimulation of the worm, and the mean ± s.e.m. 1-octanol response latency, respectively. Values of deprived worms that were different ($P < 0.05$, two-tailed Student's t-test) from stage-matched controls at the same time point are designated with an asterisk. Values that were greater than the baseline 1-octanol response latencies were tested with a one-tailed Student's t-test with unequal variance, and the value that is significantly different from the baseline response at $P < 0.05$ is designated with an arrowhead. Blue line, worms in lethargus that were not deprived of quiescence; green line, worms in lethargus that were deprived for 20 min; red line, worms in lethargus that were deprived for 30 min; black dashed line, adult worms that were not deprived; black solid line, adult worms that were deprived for 30 min. $n = 10$ for each group.

To identify genetic regulators of lethargus, we initially focused on *egl-4*. The mutant *egl-4(ad450sd)*, which contains a gain-of-function (gf) mutation in a cGMP-dependent protein kinase (PKG)[12], has been noted during its adult stage to stop moving and feeding[12,13]—behaviours normally observed to stop during lethargus. We measured the quiescence associated with lethargus in the *egl-4(gf)* mutant as well as in the *egl-4* loss-of-function (lf) null mutant *egl-4(n479)* (ref. 14). The *egl-4(gf)* mutants showed a time-dependent increase in behavioural quiescence, whereas the *egl-4(lf)* mutants showed reduced behavioural quiescence (Fig. 4a, b). *egl-4(gf)* mutants have quiescence outside of lethargus, during the normally active periods (Fig. 4a, b).

The increased behavioural quiescence of *egl-4(gf)* adults is associated with a longer latency of response to 1-octanol (Fig. 4c), indicating that the behavioural state in adults also has sleep-like properties. After strong mechanical stimulation of *egl-4(gf)* adults, the mutants resume normal adult locomotion[12,13] and respond normally to 1-octanol (Fig. 4c), indicating that they are capable of a normal sensory response. Thus, the increased 1-octanol response latency is a result of sleep-like behaviour of this mutant during the adult stage. The 1-octanol response latency of third-day adult *egl-4(gf)* worms that had been treated with *egl-4(RNAi)* for two days was shorter (7 ± 2 s) than that of control *egl-4(gf)* worms treated with control RNA interference (RNAi) (19 ± 5 s, mean ± s.e.m.; $n = 15$ for each group, $P = 0.02$, two-tailed Student's *t*-test), indicating that the sleep-like properties of *egl-4(gf)* are not the result of altered development.

In addition to implicating *egl-4* in the control of the sleep-like behaviour in *C. elegans*, the finding that sleep-like behaviours in the *egl-4(gf)* mutants can occur during the adult stage, after completion of all moults, indicates that sleep-like behaviour can be uncoupled from the moulting cycle.

In contrast to the behaviour of *egl-4(gf)*, response latency to 1-octanol during lethargus is reduced in the *egl-4(lf)* mutant (Fig. 4d), indicating a reduction in sleep-like behaviour in this mutant. Rescue of the short 1-octanol response latency of *egl-4(lf)* during lethargus was achieved by expression of *egl-4* in a subset of sensory neurons under the control of either the *odr-3* or the *tax-4* promoter but not under the control of the *odr-1* promoter (Supplementary Fig. 3). These transgenic experiments indicate that sensory neurons have a role in the regulation of lethargus. In addition, because the *odr-1* promoter used in this experiment is known to promote expression in the only two neurons that share *tax-4* and *odr-3* expression (Supplementary Table 2), these results indicate that *egl-4* can function in multiple sensory neurons to promote sleep-like behaviour. Given the demonstrated role for *egl-4* in sensory adaptation[15], one interpretation of this function in sensory neurons in regulating lethargus is that *egl-4* serves to reduce the arousal state of the animal by dampening sensory input.

The effects of *egl-4* mutations on state-dependent 1-octanol responses cannot be explained as a non-specific effect of overall activity of these mutants. This is because *egl-8* mutant adults, which like *egl-4(gf)* show decreased movement when unperturbed[16], have normal 1-octanol response latencies (Fig. 4c), and *goa-1* mutants, which

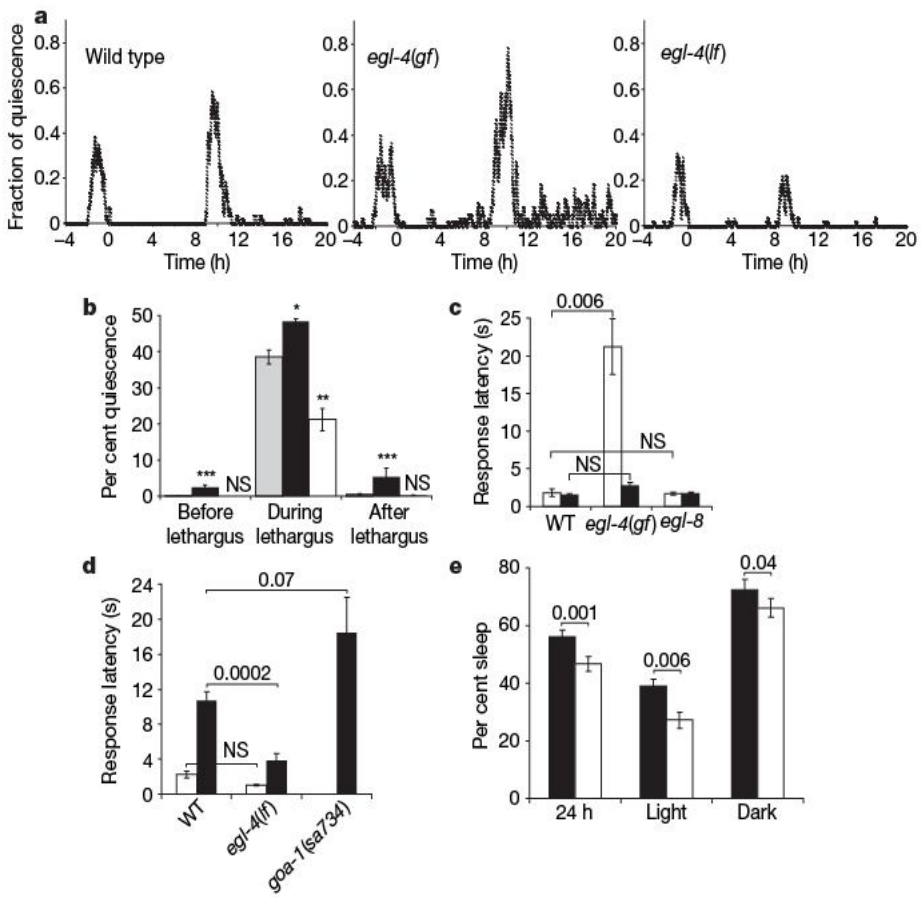

Figure 4 | The *egl-4* cGMP-dependent protein kinase promotes sleep-like behaviour. **a**, Quiescence measurement of a wild-type worm, the *egl-4(gf)* mutant *ad450* and the *egl-4(lf)* mutant *n479*. The zero time point represents the end of the L3 lethargus period. The *egl-4(gf)* mutant shows increased L4 quiescence as well as quiescence during the adult stage, whereas the *egl-4(lf)* mutant shows a reduction of behavioural quiescence associated with the L3 and L4 lethargus stages. **b**, Comparison of mean ± s.e.m. percentage quiescence in one hour in wild type (grey), *egl-4(gf)* (black) and *egl-4(lf)* (white) worms. 'Before lethargus', 'During lethargus' and 'After lethargus' are defined in Supplementary Table 1. Differences between mutant and wild type are designated $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$ (Student's *t*-test). 'NS' denotes $P > 0.1$. $n = 7$ for each mutant and $n = 21$ for wild type. **c**, Adult *egl-4(gf)* mutants, when unperturbed, show sleep-like behaviour in their response to 30% 1-octanol (white), whereas *egl-8* mutants do not. Ten minutes after strong stimulation of the animal (black), the latency is not different from that of wild-type adult worms. Error bars represent s.e.m. Comparisons were made between genotypes using a two-tailed Student's *t*-test with unequal variance. 'NS' denotes $P > 0.1$. $n = 15$ worms for each condition. **d**, During lethargus (black), *egl-4(lf)* mutants show a reduction in 1-octanol response latencies, whereas *goa-1(sa734)* mutants do not. During the L4 stage before lethargus (white), the response latencies are not different between the genotypes. Values are mean ± s.e.m. Comparisons were made using a two-tailed Student's *t*-test with unequal variance. $n = 15$ worms for each condition. **e**, Increased activity of the cGMP-dependent protein kinase gene *foraging* is associated with increased sleep in *Drosophila*. Shown is the mean ± s.e.m. percentage time spent asleep during a two-day video recording of 24–26 for^{s2} (white) and for^{R} (black) flies in the 24-h period, in the 12-h light period (Light) and in the 12-h dark period (Dark). Comparisons were made using a two-tailed Student's *t*-test with unequal variance.

like *egl-4(lf)* show increased movement outside of lethargus[17], have normal response latencies during lethargus (Fig. 4d). The timing of lethargus, as reflected by the duration between the quiescence peaks of the L3 and L4 lethargus, was not different in wild-type (11.3 ± 0.2 h, $n = 30$), *egl-4(gf)* (11.1 ± 0.6 h, $n = 6$) and *egl-4(lf)* (11.4 ± 0.5 h, $n = 5$) worms, indicating that *egl-4* affects the expression and not the timing of sleep-like behaviour.

To test for the possibility that the quiescence-promoting effects of PKG are phylogenetically conserved, we compared sleep in *D. melanogaster* strains that differed in the activity of the *foraging* (*for*) gene, which encodes a *Drosophila* PKG[18] similar in sequence and function to *egl-4* (ref. 19; Supplementary Information). Cyclic GMP has previously been implicated in the signalling events that control insect pre-ecdysis behaviour[20]. We found that the *Drosophila* strain for^{s2}, which has low PKG levels, slept less than the for^{R} strain from which it was derived[21] (Fig. 4e). Therefore, as in *C. elegans*, greater PKG activity is associated with more sleep in *Drosophila*.

To explore further the idea that there is some conservation of genetic regulation of sleep-like behaviour, we studied worms that carry reduction-of-function mutations in *pde-4* and worms that carry a gain-of-function mutation in *acy-1*—genes that encode *C. elegans* homologues of *Drosophila dunce*[22] and *rutabaga*[23], respectively. Studies of *dunce* and *rutabaga* have led to the conclusion that cAMP signalling promotes *Drosophila* wakefulness[24]. The 1-octanol response latency during lethargus of *pde-4* and *acy-1* mutants was reduced (Supplementary Fig. 4). This increased sensory responsiveness during a normally sleep-like period suggests that cAMP signalling antagonizes worm sleep-like behaviour. These effects of *pde-4*, *acy-1* and *egl-4* mutations in *C. elegans* indicate that there is some conservation in the genetic control of sleep. Independent evidence of such conservation was reported recently[25].

The reason for the evolution of sleep is unknown. The temporal relationship between *C. elegans* lethargus and the moult, which is required for animal growth and development and is a time of biosynthetic activity[26,27], suggests that this sleep-like state has a role in growth and development. Synaptic changes occur during a lethargus period[28,29], suggesting that lethargus promotes nervous system change. A role in nervous system development is interesting in light of data suggesting that sleep is necessary for changes in the nervous system[30].

METHODS SUMMARY

A digital video analysis method based on a frame subtraction principle was used to identify 10-s epochs of behavioural quiescence. This method has a spatial resolution for movement detection that approaches 5 μm. Additional details of this method as well as methods for deprivation of quiescence are described in the Methods. *C. elegans* strains used as well as methods for sensory stimulation, for statistical analysis, for *Drosophila* sleep measurements, for transgenesis and for RNAi are detailed in the Supplementary Information.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions D.M.R. designed and performed research, J.E.Z. performed *Drosophila* experiments, M.H.M. wrote computer programs, U.D.T. performed behavioural experiments involving 1-octanol response measurements, Y.Y. showed that *tax-4p::egl-4* can rescue sleep-like behaviours of *egl-4(lf)*, and M.V.S. and A.I.P. provided input into research design and drafted this manuscript along with D.M.R.

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LETTERS

NLRX1 is a regulator of mitochondrial antiviral immunity

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The RIG-like helicase (RLH) family of intracellular receptors detect viral nucleic acid and signal through the mitochondrial antiviral signalling adaptor MAVS (also known as Cardif, VISA and IPS-1) during a viral infection[1–6]. MAVS activation leads to the rapid production of antiviral cytokines, including type 1 interferons. Although MAVS is vital to antiviral immunity, its regulation from within the mitochondria remains unknown. Here we describe human NLRX1, a highly conserved nucleotide-binding domain (NBD)- and leucine-rich-repeat (LRR)-containing family member (known as NLR) that localizes to the mitochondrial outer membrane and interacts with MAVS. Expression of NLRX1 results in the potent inhibition of RLH- and MAVS-mediated interferon-β promoter activity and in the disruption of virus-induced RLH–MAVS interactions. Depletion of NLRX1 with small interference RNA promotes virus-induced type I interferon production and decreases viral replication. This work identifies NLRX1 as a check against mitochondrial antiviral responses and represents an intersection of three ancient cellular processes: NLR signalling, intracellular virus detection and the use of mitochondria as a platform for anti-pathogen signalling. This represents a conceptual advance, in that NLRX1 is a modulator of pathogen-associated molecular pattern receptors rather than a receptor, and identifies a key therapeutic target for enhancing antiviral responses.

Mammalian members of the nucleotide-binding domain (NBD) and leucine-rich-repeat-containing (LRR) (known as NLR, see http://www.genenames.org/genefamily/nacht.html) family of proteins are indispensable for cellular responses to pathogens. This NBD-LRR protein structure is ancient and highly conserved, as shown by its initial identification among plant disease-resistance proteins[7–12]. Current dogma posits that NLRs function as cytoplasmic surveillance molecules that sense intracellular pathogen-associated molecular patterns (PAMPs), or as regulators of pathogen-initiated signalling cascades[13,14]. Viral PAMPs are detected by the cytoplasmic RLH receptors RIG-I (also known as DDX58) and MDA-5 (also known as IFIH1), which signal through the mitochondrial protein MAVS, resulting in the activation of interferon regulatory factor 3 (IRF3) and NF-κB and type-1 interferon transcription[1–6]. Abrogation of MAVS expression or function leads to reduced type 1 interferon production and antiviral protection[15].

To study the potential role of NLR proteins in regulating mitochondrial antiviral signalling, we used bioinformatics to identify NLRs localized to the mitochondria. We identified one putative mitochondrial NLR called NLRX1 (previously known as CLR11.3 and NOD9)[9,16] (Fig. 1a). The predicted peptide sequence and distinct domains of NLRX1 are shown in Supplementary Fig. 1. Consistent with the conserved motif structure of the NLR family, NLRX1 contains a central putative NBD and carboxy-terminal LRRs. The assignment of the amino-terminal effector domain to a subclass is less clear. Instead, the N terminus has evolved to include a mitochondrial-targeting sequence (Supplementary Fig. 1). On the basis of hydrophobicity analyses, we identified two putative transmembrane regions (Supplementary Fig. 2). NLRX1 homologues were identified in all vertebrates examined, with a remarkably high (92.4%) degree of conservation between human and mouse (Supplementary Fig. 3).

To investigate NLRX1 function, NLRX1 was isolated and the complementary DNAs encoding N and C termini were verified by rapid amplification of cDNA ends (RACE). The 3′ RACE produced two products, identifying a splice variant lacking a portion of the LRR region encoded by exon 9 (Supplementary Fig. 4). *NLRX1* messenger RNA is broadly expressed, suggesting a ubiquitous role (Supplementary Fig. 5). To examine protein expression, we generated an anti-NLRX1 monoclonal antibody. Antibody specificity was verified by immunoblotting against transfected, epitope-tagged haemagglutinin (HA)–NLRX1 or another NLR protein HA–NLRP12 (also known as Monarch1; ref. 17). NLRX1 antibody detected a strong band corresponding to the exogenous HA-tagged NLRX1 (Supplementary Fig. 6) and a weaker endogenous product of similar size in cells transfected with pcDNA or with HA-tagged NLRP12 (Supplementary Fig. 6). Short interfering RNA (siRNA)-mediated knockdown of *NLRX1* greatly reduced the detection of this endogenous product, whereas control siRNA oligonucleotides had no effect (Supplementary Fig. 7). NLRX1 protein was also detected in all cell lines analysed, further suggesting ubiquitous expression (Supplementary Fig. 8).

Additional clues to NLRX1 function are revealed by its cellular localization. Both endogenous and overexpressed NLRX1 revealed a punctate cytoplasmic distribution (Fig. 1b and Supplementary Fig. 9a). Biochemical fractionation verified that endogenous NLRX1 protein resided in the non-nuclear fraction (Fig. 1c, middle panel). This fraction contains both cytosol and cytoplasmic organelles, the latter of which include the mitochondria. Separation of this cytoplasmic/organellar fraction into three fractions (small membranes, soluble cytosol and mitochondria) revealed that NLRX1 was found exclusively in the mitochondrial fraction, which also contained the mitochondrial protein TOM20 (translocase of outer mitochondrial membrane 20 homologue; also known as TOMM20) (Fig. 1c, right panel). A merged confocal image of endogenous NLRX1 and the mitochondrial stain Mitotracker indicated mitochondrial localization of NLRX1 (Supplementary Fig. 9b). Antibody staining by immunogold-bead electron microscopy was used as the ultimate method for visualizing NLRX1; this localized NLRX1 to the

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mitochondria with little staining elsewhere (Fig. 1d, top panels). Co-staining for NLRX1 (small beads) and MAVS (large beads) identified several mitochondria containing both proteins (Fig. 1d, middle panels). Isotype control antibody showed no nonspecific staining (Fig. 1d, bottom left panel), whereas the positive control TOM20 showed mitochondrial staining (Fig. 1d, bottom right panel). As observed for MAVS and TOM20, many of the NLRX1-specific immunogold beads were proximal to the mitochondrial membrane. To study this further, we separated mitochondrial membrane fractions by a sucrose gradient (Fig. 1e). Voltage-dependent anion channel 1 (VDAC1) and MAVS are proteins known to reside in the mitochondrial outer membrane and served as positive controls[1,18]. VDAC1, MAVS and NLRX1 were the only proteins detected in fractions 1–5, which includes primarily mitochondrial outer membrane proteins (Fig. 1e). COXIV (cytochrome c oxidase subunit IV isoform 1; also known as COX4I1) is an inner membrane mitochondrial protein and was only found in fractions 6–10. To confirm this finding, a trypsin protection assay was performed on prepared mitochondrial fractions. NLRX1 and the known outer membrane proteins MAVS and BCL2L1 were all sensitive to proteolytic cleavage by trypsin, whereas the mitochondrial inner membrane protein COXIV was completely protected (Fig. 1f). Furthermore, deletion of the N-terminal mitochondrial targeting sequence resulted in a loss of mitochondrial localization (Supplementary Fig. 10). These results indicate that NLRX1 resides in the outer mitochondrial membrane.We next examined the interaction of NLRX1 and MAVS. Co-immunoprecipitation studies demonstrate that HA–NLRX1 interacts with MAVS (Fig. 2a) but not with other known mitochondrial outer membrane proteins (BCL2 and BCL2L1), indicating specificity of the NLRX1–MAVS interaction (Fig. 2b). Finally, endogenous NLRX1 associates strongly with endogenous MAVS after immunoprecipitation with two different MAVS antibodies (Fig. 2c). Consistent with these results, *MAVS* and *NLRX1* show a remarkably similar expression level in many cell types (Supplementary Fig. 11).

NLRX1 and MAVS are both modular proteins; therefore, we sought to investigate the specific domains required for this interaction. The

a

Gene	Mitochondria	Cytosol	Prediction
NLRX1 (CLR11.3/NOD9)	52%	22%	Mito.
NLRP12 (Monarch1/CLR19.3/PYPAF7/NALP12/PAN6)	26%	35%	Cytosol
NLRP2 (CLR19.9/NBS1/PYPAF2/NALP2)	17%	48%	Cytosol
NLRP3 (CIAS1/NALP3/Pypaf1)	17%	39%	Cytosol
NLRP4 (CLR19.5/RNH2/PYPAF4/NALP4/PAN2)	17%	35%	Cytosol
NLRP6 (CLR11.4/PAN3/PYPAF5/NALP6)	17%	35%	Cytosol
NLRP13 (CLR19.7/NOD14/PAN13/NALP13)	17%	48%	Cytosol
NAIP (BIRC1/CLR5.1/NLRB1)	17%	52%	Cytosol
NLRP14 (CLR11.2/NOD5/NALP14/PAN8)	13%	39%	Cytosol
CIITA (NLRA/MHCC2TA)	13%	26%	Nuclear
NLRP9 (CLR19.1/NOD6/NALP9/PAN12)	9%	91%	Cytosol
NOD2 (CARD15/CLR16.3/NLRC2)	9%	52%	Cytosol
NLRP7 (CLR19.4/Nod12/NALP7/PAN7)	4%	52%	Cytosol
NLRP10 (CLR11.1/NOD8/NALP10/PAN5/PYNOD)	4%	61%	Cytosol
NLRC3 (CLR16.2/NOD3)	4%	48%	Cytosol
NLRC4 (IPAF/CARD12/CLR2.1)	0%	52%	Cytosol

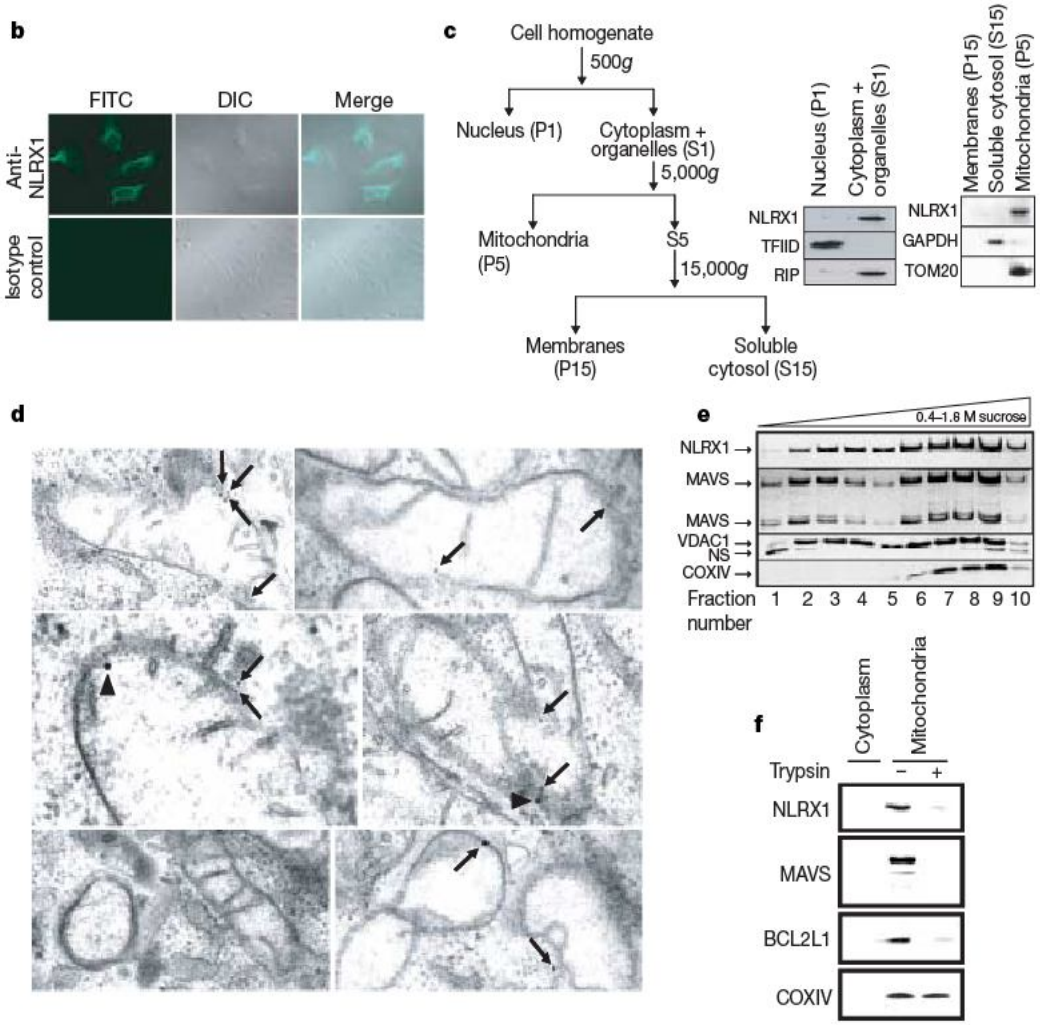

Figure 1 | NLRX1 is an outer membrane mitochondrial protein. **a**, Prediction of subcellular localizations for NLRs. This was calculated using K-nearest neighbours (k-NN) classifier predictions of representative NLR localizations. Listed are the official gene symbols followed by aliases in parentheses. **b**, Cytoplasmic localization of endogenous NLRX1. FITC, fluorescein isothiocyanate; DIC, differential interference contrast. **c**, Left, fractionation scheme used. Right, immunoblot of NLRX1 in various cell fractions. **d**, Immunogold-bead electron micrographs of NLRX1 alone (top panels), NLRX1 (small bead, arrow) and MAVS (large bead, arrowhead) (middle panels), isotype antibody control (bottom left panel), and TOM20 (bottom right panel). **e**, Immunoblotting of NLRX1 and known outer (VDAC1 and MAVS) and inner (COXIV) mitochondrial proteins in membrane fractions. NS denotes nonspecific protein band. **f**, Trypsin treatments of mitochondria followed by immunoblotting with NLRX1, MAVS and BCL2L1(outer membrane), as well as COXIV (inner membrane).

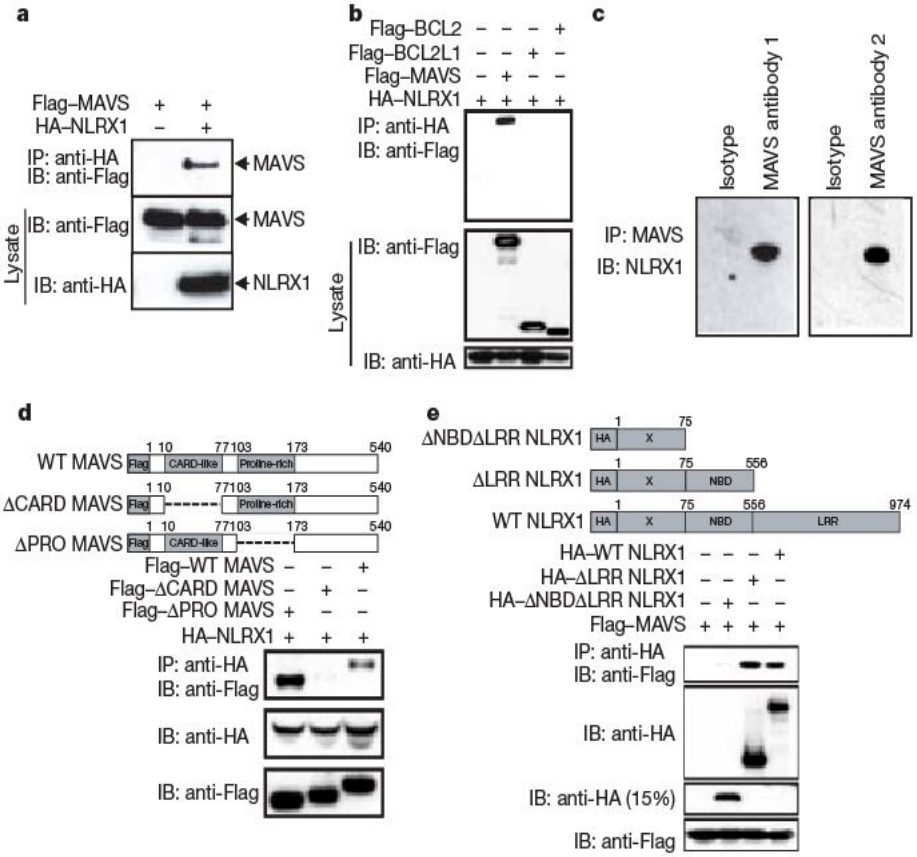

Figure 2 | NLRX1 interacts with MAVS. **a**, Interaction of overexpressed NLRX1 and MAVS. IP and IB denote immunoprecipitation and immunoblotting, respectively. **b**, Interactions of NLRX1 with other known mitochondrial outer membrane proteins. **c**, Interaction of endogenous NLRX1 with endogenous MAVS. **d**, Interaction of MAVS truncation mutants with wild-type (WT) NLRX1. **e**, Interaction of NLRX1 truncation mutants with wild-type MAVS. Deletion constructs are shown above; 15% denotes 15% polyacrylamide gel necessary to resolve the smallest protein. The X domain is currently undefined.

N-terminal CARD domain of MAVS is required for MAVS signalling and interactions with RLH family members[1]. It is also required for the interaction with NLRX1 (Fig. 2d). In NLRX1, deletion of the LRR had no effect on the NLRX1–MAVS interaction. A further deletion of the putative NBD disrupted this interaction, indicating that this domain is required for maintaining the interaction with MAVS (Fig. 2e). Collectively, these data demonstrate that NLRX1 is a MAVS-associated mitochondrial outer membrane protein and raise the intriguing possibility that it participates in antiviral signalling.

Signalling in response to the synthetic viral double-stranded RNA (dsRNA) analogue poly(I:C) is mediated both at the cell membrane through Toll-like receptor 3 (TLR3) and within the cytoplasm by means of direct binding to the RLH molecules to activate MAVS. Both pathways activate NF-κB and IRF3, leading to interferon β (IFN-β) transcription[5]. Poly(I:C), cannot activate IFN-β expression in TLR3-deficient 293T cells when applied extracellularly. However, when delivered into the cytoplasm by transfection, poly(I:C) stimulates IFN-β through the MAVS pathway. Conversely, stable TLR3-expressing 293T cells have a robust response to extracellular poly(I:C), which does not require MAVS signalling. We used these two cell lines in poly(I:C)-induced IFN-β luciferase experiments to delineate a role for NLRX1 in intracellular (RLH) versus extracellular (TLR) antiviral responses. NLRX1 had no effect on the TLR3-mediated extracellular activation of IFN-β or NF-κB luciferase repor-

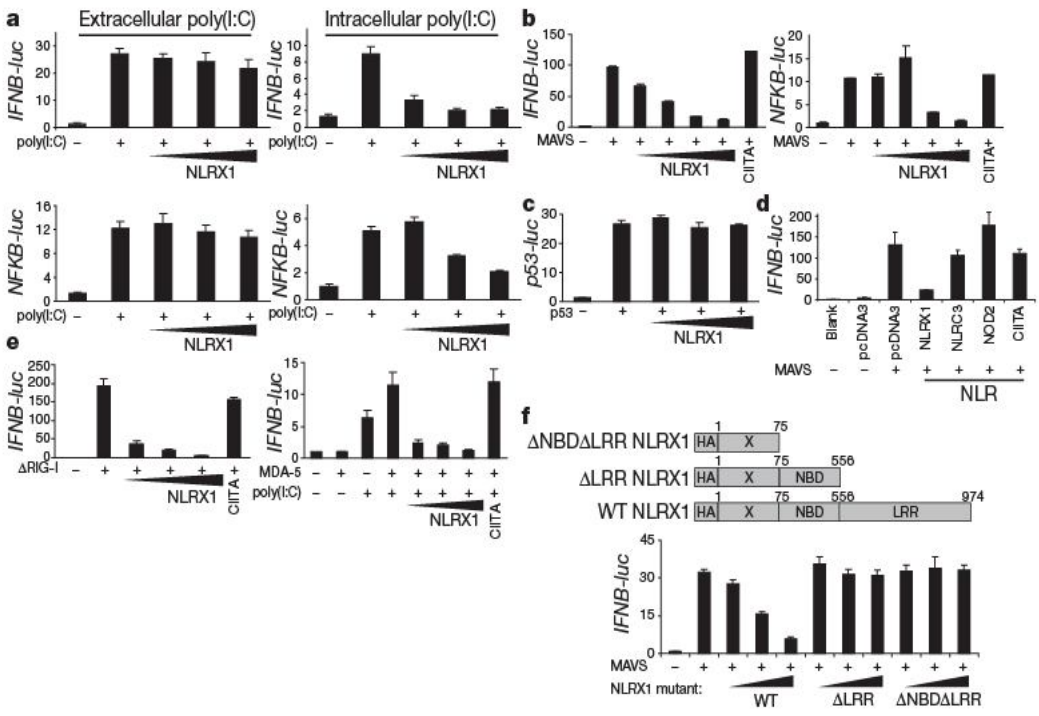

Figure 3 | NLRX1 inhibits MAVS-mediated activation of IFN-β and NF-κB. **a–c**, Effect of NLRX1 on *IFNB*- and *NFKB*-luciferase (*luc*) activation by extracellular and intracellular poly(I:C) (**a**); *IFNB-luc* (left) and *NFKB-luc* (right) activation by MAVS (**b**); and p53 activity (**c**). **d**, *IFNB-luc* is inhibited by NLRX1 but not by other NLR proteins. **e**, Effect of NLRX1 on ΔRIG-I- (left) and MDA-5- (right) mediated activation of *IFNB-luc* activity. **f**, Repression of MAVS-mediated *IFNB-luc* by wild-type (WT) NLRX1 compared with its truncation mutants. Data from **a–f** are presented as mean ± s.d. from three independent experiments.

ters (Fig. 3a, top and bottom left panels). By contrast, the intracellular IFN-β response was strongly inhibited by NLRX1 (Fig. 3a, top right panel). NF-κB luciferase reporter activation was likewise reduced, although to a lesser extent (Fig. 3a, bottom right panel). Thus, NLRX1 acts as a negative regulator within the intracellular antiviral pathway.

We next sought to study MAVS activation of IFN-β directly. MAVS potently activated the IFN-β reporter luciferase, and NLRX1 inhibited this activation in a dose-dependent fashion (Fig. 3b, left). MAVS-induced NF-κB luciferase activity was inhibited by NLRX1, but to a lesser extent (Fig. 3b, right). As a specificity control, p53 luciferase reporter was not affected by NLRX1 (Fig. 3c). Other NLR proteins (CIITA, NOD2 and NLRC3 (also known as CLR16.2)) did not significantly affect IFN-β luciferase (Fig. 3d). Expression of the constitutively active truncation mutant Δ*RIG-I* induces a robust intracellular antiviral response through MAVS[19,20]. NLRX1 significantly reduced the ability of ΔRIG-I to activate IFN-β transcription (Fig. 3e, left). MDA-5 is the intracellular receptor for poly(I:C) and picornavirus nucleic acid and also requires MAVS[6]. NLRX1 also abolished MDA-5 activity (Fig. 3e, right). Furthermore, mapping of the NLRX1 functional domain revealed that the C-terminal LRR domain is required for the repression of MAVS-induced interferon signalling (Fig. 3f). These data indicate that NLRX1 functions as an inhibitor of RLH-mediated MAVS antiviral signalling through the NLRX1 LRR domain.

To confirm that endogenous NLRX1 also repressed IFN-β production, *NLRX1*-specific siRNA oligonucleotides, which reduced NLRX1 protein by greater than 90% (Supplementary Fig. 7), were used. As expected, *IFNB* and *IFNA* mRNA levels rose sharply by 16 h post-transfection with MAVS. Both interferons were significantly increased in cells containing *NLRX1* siRNA (Fig. 4a). This increased MAVS-mediated response was also observed with the NF-κB-responsive genes interleukin 6 (*IL6*), *RANTES* (also known as *CCL5*) and tumour necrosis factor α (*TNFa*), although to a lesser extent, consistent with the aforementioned NF-κB luciferase data (Fig. 4a). To test the effect of NLRX1 during a viral infection, *IFNB* mRNA and protein levels were quantified in NLRX1-deficient cells infected with Sendai virus, which activates RIG-I and MAVS for type-1 interferon production[1]. *IFNB* mRNA induction by Sendai virus was increased >8-fold in cells with *NLRX1* siRNA compared with control siRNA (Fig. 4b, left). Consistent with the transcriptional data, the Sendai-virus-induced IFN-β protein level was also greater in the infected *NLRX1* siRNA cells (Fig. 4b, right). In addition, NLRX1 expression attenuated MAVS, ΔRIG-I and Sendai-virus-induced IRF3 dimer formation, indicating that NLRX1 reduced MAVS-mediated IRF3 signalling (Fig. 4c).

To identify a mechanism for the inhibitory effect of NLRX1 on antiviral signalling, we examined the effect of NLRX1 on the endogenous association of MAVS with RIG-I. The interaction of MAVS and RIG-I was enhanced by Sendai viral infection, whereas the introduction of NLRX1 eliminated this enhancement (Fig. 4d). Because this RLH–MAVS interaction is required for signalling, the disruption of this interaction by NLRX1 should squelch further downstream antiviral signalling. We extended these results by measuring cell death, viral replication and IFN-β production in *NLRX1* siRNA cells infected with the human alphavirus Sindbis. In congruence with the Sendai-virus findings, *NLRX1* siRNA cells infected with Sindbis produced more IFN-β protein than control siRNA cells (Fig. 4e, left) and the cells were highly resistant to green fluorescent protein (GFP)–Sindbis viral replication (25.06%) compared with the control siRNA cells (83.87%) (Fig. 4e, right). The differences in cell death are not significant and therefore can not account for the large difference in viral susceptibility (Supplementary Fig. 12). Cumulatively, these observations solidify the negative regulatory role of NLRX1 in MAVS-mediated antiviral responses, and demonstrate that it is mediated through the inhibition of virus-induced RLH–MAVS interactions.

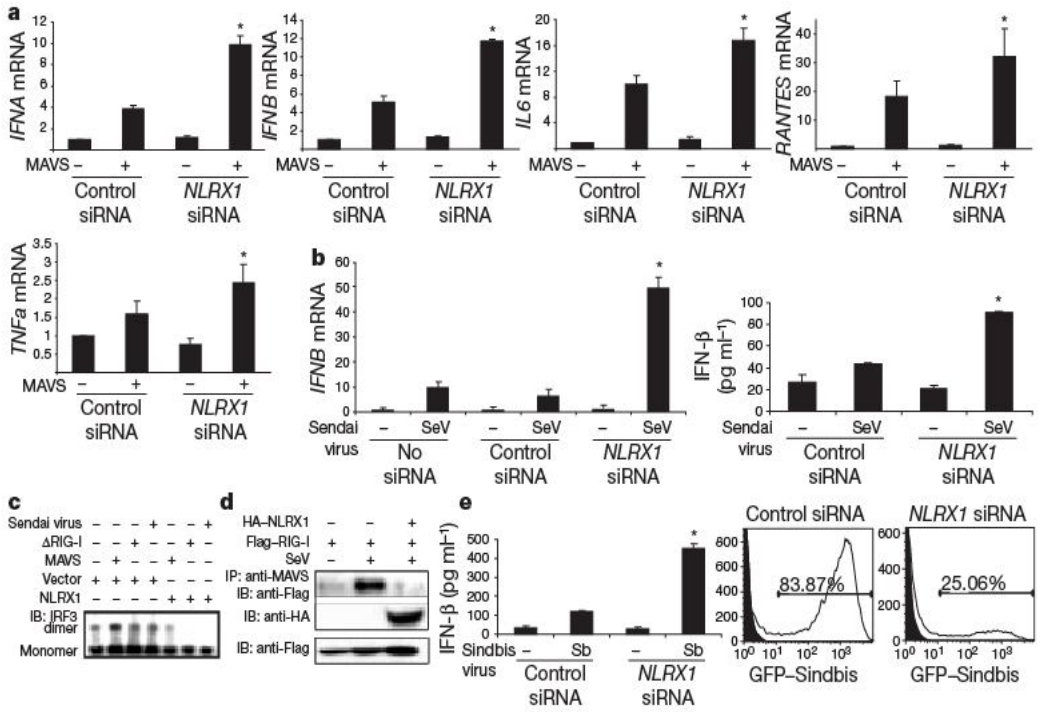

Figure 4 | *NLRX1* siRNA results in enhanced antiviral responses. **a**, Activation of *IFNB*, *IFNA* and the NF-κB responsive genes *IL6*, *RANTES* and *TNFa* by MAVS in the presence of control or *NLRX1*-targeted siRNA as measured by quantitative real-time PCR. All results were normalized to 18S rRNA values. **b**, *IFNB* mRNA (left) and protein (right) in Sendai-virus (SeV)-infected HEK293T cells in the presence of control non-targeting or *NLRX1*-targeted siRNA. Data from **a** and **b** are presented as means ± s.d. from three independent experiments. **c**, Immunoblotting of IRF3 in native whole-cell lysates from MAVS, ΔRIG-I and Sendai-virus-infected cells after overexpression of empty vector or NLRX1. **d**, Co-immunoprecipitation of endogenous MAVS with overexpressed RIG-I following mock or Sendai virus infections in the presence or absence of overexpressed NLRX1. **e**, Left panel, IFN-β protein levels from Sindbis-virus-infected 293T cells containing control or *NLRX1* siRNA. Data are presented as means ± s.d. of triplicate determinations from two independent experiments. Right panel, percentage of GFP–Sindbis-positive cells in 293T cells containing control or *NLRX1* siRNA as analysed by flow cytometry.

Interestingly, *NLRX1* mRNA and protein levels are stable after activation of MAVS signalling (Supplementary Fig. 13); therefore, the regulation of NLRX1 apparently does not involve simple transcriptional or translational mechanisms. This report shows a linkage between the rapidly emerging NLR family and mitochondrial antiviral signalling. We speculate that the mitochondrial membrane provides a convenient surface on which antiviral cell-signalling complexes are arranged and activated. Rather than directly engaging a pathogen-derived product, NLRX1 seems to function as a modulator of PAMP receptors (MOPR) by affecting the interaction of the pathogen receptor RIG-I (and possibly MDA-5) with MAVS. This mechanism is more in-line with the Guard hypothesis proposed for the indirect detection of pathogen products by plant R proteins[21,22]. Because both NLRX1 and RIG-I interact with the CARD domain of MAVS, a probable scenario is that NLRX1 and RIG-I compete for binding with the CARD domain of MAVS, with opposing outcomes. Because MAVS is a potent activator of type 1 interferon, a brake within this mitochondrial signalling complex could prevent unwarranted deleterious antiviral responses and regulate interferon signalling during the course of a viral infection. This report shows that NLRX1 is such a brake. It further demonstrates that targeting of NLRX1 through approaches such as siRNA could enhance antiviral responses, which has broad implications for the treatment of viral-associated diseases.

METHODS SUMMARY

Cloning of *NLRX1*. A complete open reading frame of human *NLRX1* was isolated from Jurkat cDNA using standard PCR techniques. The complete N and C terminals were verified by RACE.

Expression data and antibody production. Expression data for *NLRX1* were mined from the Genomics Institute of the Novartis Research Foundation. Recombinant hexahistidine-tagged NLRX1 was used as an antigen for the production of monoclonal antibody. NLRX1 antigen was injected into mice and monoclonal antibodies were produced by standard methodology.

Real-time PCR. First-strand cDNA was generated from total RNA using random priming and moloney murine leukemia virus (MMLV) reverse transcriptase (Invitrogen). Real-time PCR was performed using QuantiTect SYBR Green PCR Master Mix (Qiagen) in triplicate experiments and analysed on an AB Prism 7700 analyser (Applied Biosystems). All real-time values were normalized to 18S ribosomal RNA.

Immunofluorescent staining, immunogold and mitochondrial localizations. HeLa cells were transfected with 1.0 μg per well GFP–NLRX1 or stained with NLRX1 antibody. Anti-mouse Alexa Fluor 488 (Molecular Probes) was used to stain for endogenous NLRX1. All exogenous and endogenous NLRX1 localizations were visualized on a Zeiss LSM5 PASCAL confocal microscope. To determine co-localization with mitochondria, HeLa cells were co-stained with Mitotracker (Invitrogen) and endogenous NLRX1. Transmission electron microscopy of anti-NLRX1 immunogold labelling was also performed by standard methods. NLRX1 protein was detected in sucrose gradient fractions and trypsin-treated mitochondrial fractions.

Luciferase assays, co-immunoprecipitations and siRNA. HEK293T or TLR3-HEK293T cells were transfected in 96-well plates with IFN-β, NF-κB or p53 luciferase constructs and HA–NLRX1. Exogenous MAVS, poly(I:C) or p53 plasmid was used as an agonist. All co-immunoprecipitation studies of exogenous and endogenous proteins were performed from whole cells lysed in RIPA buffer and following standard methodology. Non-targeting, glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) or *NLRX1* siRNA oligonucleotides (Dharmacon) were transfected following the commercial protocol.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Contributions C.B.M. and J.P.-Y.T. designed the experiments and prepared the manuscript. C.B.M. performed the experiments. D.T.B. performed several experiments and assisted with the manuscript. J.A.D. performed cell fractionation studies and produced antigen for antibody production. Y.L. tested antibody specificity and provided considerable technical assistance. M.T.H. and T.E.M. assisted with all viral studies. A.G.Z. created NLRX1 truncation mutants. M.A.A-L. produced monoclonal antibody and provided several hybridomas for analysis. V.J.M. performed immunogold-bead transmission electron microscopy. L.S. performed mitochondrial fractionation by sucrose gradient. Z.Y. performed flow cytometry, and J.D.L. assisted with co-immunoprecipitation studies.

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LETTERS

Macrophage migration inhibitory factor stimulates AMP-activated protein kinase in the ischaemic heart

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Understanding cellular response to environmental stress has broad implications for human disease. AMP-activated protein kinase (AMPK) orchestrates the regulation of energy-generating and -consuming pathways, and protects the heart against ischaemic injury and apoptosis[1]. A role for circulating hormones such as adiponectin[2] and leptin[3] in the activation of AMPK has received recent attention. Whether local autocrine and paracrine factors within target organs such as the heart modulate AMPK is unknown. Here we show that macrophage migration inhibitory factor (MIF), an upstream regulator of inflammation[4], is released in the ischaemic heart, where it stimulates AMPK activation through CD74, promotes glucose uptake and protects the heart during ischaemia-reperfusion injury. Germline deletion of the *Mif* gene impairs ischaemic AMPK signalling in the mouse heart. Human fibroblasts with a low-activity *MIF* promoter polymorphism[5] have diminished MIF release and AMPK activation during hypoxia. Thus, MIF modulates the activation of the cardioprotective AMPK pathway during ischaemia, functionally linking inflammation and metabolism in the heart. We anticipate that genetic variation in *MIF* expression may impact on the response of the human heart to ischaemia by the AMPK pathway, and that diagnostic *MIF* genotyping might predict risk in patients with coronary artery disease.

Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine that controls the inflammatory 'set point' by regulating the release of other pro-inflammatory cytokines[6]. MIF is expressed in several cell types, including monocytes/macrophages[7], vascular smooth muscle[8] and cardiomyocytes[9], and is released on stimulation from pre-formed storage pools. MIF is involved in the pathogenesis of inflammatory diseases, such as atherosclerosis[8,10], rheumatoid arthritis[5], sepsis[4], asthma[11] and acute respiratory distress syndrome[12]. Human *MIF* gene expression is determined by promoter polymorphisms, including a tetra-nucleotide CATT repeat at position –794 (ref. 5). MIF signalling is known to activate ERK1/2 MAPK (ref. 13) through a receptor complex comprising CD74 (ref. 14) and CD44 (ref. 15). In contrast, the chemokine receptors CXCR2 and CXCR4 participate in MIF-mediated migratory function[10].

MIF also stimulates glycolysis during sepsis, increasing the synthesis of fructose 2,6-bisphosphate and cellular glucose uptake[16]. The signalling pathways by which MIF exerts its metabolic effects are unknown, but one candidate is the AMP-activated protein kinase (AMPK)—an important regulator of both glycolysis and glucose uptake during cellular stress[1]. AMPK senses the cellular energy state and affects diverse pathways to increase cellular ATP production and limit energy consumption. AMPK activity is regulated by AMP binding to its regulatory γ-subunit[17] and by phosphorylation of the catalytic α-subunit by upstream kinases, including LKB1 (ref. 18) and CaMKKβ (ref. 19). In the heart, AMPK stimulates 6-phosphofructo-2-kinase activity and glycolysis[20], induces glucose transporter-4 (GLUT4, encoded by the *SLC2A4* gene) translocation[21], increases ischaemic glucose uptake[1,22] and limits myocardial injury and apoptosis[1].

AMPK phosphorylation is also modulated by the adipocyte-derived circulating hormones leptin[3] and adiponectin[23], raising the possibility that cytokines might also activate AMPK. We hypothesized that AMPK might be activated in an autocrine/paracrine fashion by MIF in the heart during ischaemia, linking the regulatory control of inflammation and metabolism.

Initial experiments examined whether MIF has a role in the stimulation of the AMPK pathway during hypoxia in rat heart muscles. Hypoxic activation of AMPK (Fig. 1a) was associated with a twofold increase in muscle MIF release (Fig. 1b), the latter consistent with previous results in cardiomyocytes[24]. Pre-treatment with anti-MIF antibody reduced hypoxic AMPK activation by 67% (Fig. 1c). One of the important AMPK actions during hypoxia and ischaemia is to increase glucose transport[1,22]. Hypoxic glucose transport was inhibited 38% by anti-MIF antibody (Fig. 1d), indicating that secreted extracellular MIF modulates downstream AMPK action.

To investigate whether MIF modulates AMPK, we added MIF to normoxic heart muscles. MIF caused time- and dose-dependent increases in AMPK phosphorylation (Fig. 1e and f), and increased heart muscle glucose uptake (Fig. 1g). Hypoxia and insulin-stimulated glucose uptake in the heart are mediated by translocation of the glucose transporter GLUT4 to the cell surface where it is physiologically active[21]. We used a cell-membrane impermeant photolabel compound and found significant translocation of GLUT4 to the cell surface (Fig. 1h), elucidating the mechanism through which MIF increases glucose uptake.

We next examined whether MIF modulates AMPK signalling in the ischaemic heart. MIF is expressed by cardiomyocytes[9,24], endothelial cells, monocytes and macrophages[7]. We used the isolated mouse heart perfused with crystalloid buffer, eliminating the potential contribution of MIF from circulating cells. MIF was highly expressed in cardiomyocytes, according to immunohistochemical data (Fig. 2a). Ischaemia triggered cardiac MIF release into the coronary venous effluent and decreased heart MIF content after ischaemia-reperfusion (Fig. 2b).

To determine whether MIF plays a part in ischaemic AMPK activation, we used hearts from $Mif^{-/-}$ mice[25] and compared them to wild-type controls. $Mif^{-/-}$ mice demonstrated a normal baseline cardiac phenotype with respect to left ventricular size and function, histology and the expression of AMPK and glucose transporter proteins

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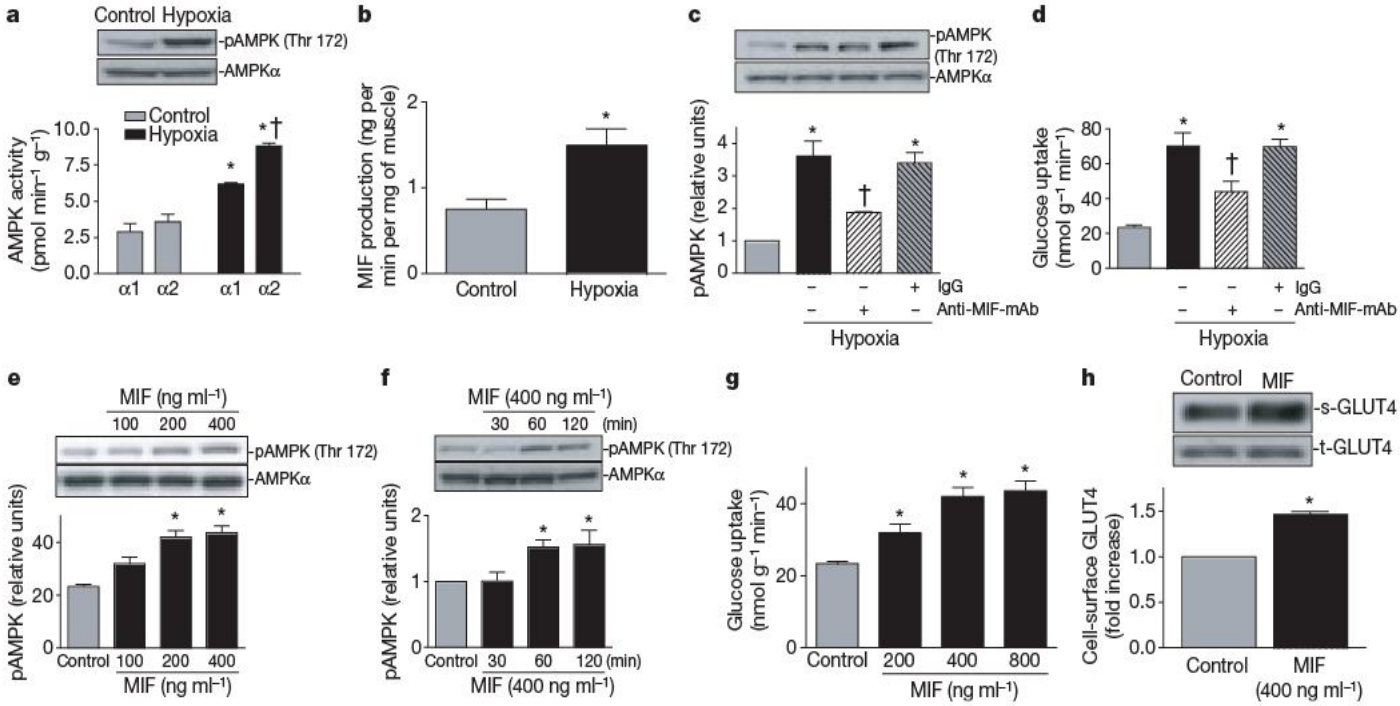

Figure 1 | Role of MIF in heart muscle AMPK signalling during hypoxia. **a**, Immunoblots show phosphorylated and total AMPK, bars show α2 or α1 AMPK activity. $*P = 0.001$, versus control; $\dagger P = 0.012$, α1 versus α2. **b**, Muscle MIF release. $*P = 0.03$, versus control. **c**, **d**, Inhibition of AMPK activation and downstream glucose transport by anti-MIF antibody (100 μg ml^{-1}). $*P = 0.02$, versus control; $\dagger P = 0.04$, versus hypoxia alone. **e**, AMPK activation by recombinant MIF (60 min). $*P < 0.02$, versus control. **f**, AMPK activation by recombinant MIF (400 ng ml^{-1}). $*P < 0.05$, versus control. **g**, Glucose uptake during incubation with recombinant MIF. $*P < 0.05$, versus control. **h**, Immunoblots of cell-surface (s-GLUT4) and total (t-GLUT4) GLUT4 in muscles incubated without or with 400 ng ml^{-1} rat MIF. $*P < 0.001$ versus control, $n = 3$–6 per group. Values are means ± s.e.m.

(Supplementary Fig. 1). When perfused with mixed-substrate buffer and subjected to 15 min of global ischaemia, AMPK activation was significantly blunted in the *Mif*$^{-/-}$ hearts owing to decreased phosphorylation of the activating Thr 172 site (Fig. 3a). The tumour-suppressor kinase LKB1 (also known as SKT11) has an important role in mediating AMPK phosphorylation during ischaemia[18]. However, we observed no change in the expression of LKB1, or CaMKKβ (also known as Camkk2), another potential upstream kinase (Supplementary Fig. 1).

Because AMPK mediates glucose uptake during ischaemia[1], we examined whether the defect in AMPK signalling in the *Mif*$^{-/-}$ hearts also diminished downstream glucose uptake. Although glucose uptake was normal in *Mif*$^{-/-}$ hearts during control perfusions, the stimulation of glucose uptake during ischaemia-reperfusion was significantly blunted compared to wild-type hearts (Fig. 3b). This was associated with impaired glycogen synthesis in *Mif*$^{-/-}$ hearts during post-ischaemic reperfusion, despite a comparable amount of glycogen breakdown during ischaemia (Supplementary Fig. 2).

Consistent with prior observations that AMPK deficiency is functionally deleterious to the heart during ischaemia-reperfusion[1], *Mif*$^{-/-}$ hearts also demonstrated impaired ischaemic tolerance (Fig. 3c). *Mif*$^{-/-}$ hearts subjected to *ex vivo* ischaemia had decreased post-ischaemic left ventricular function (Fig. 3c) as well as increased ischaemic diastolic pressure and reduced contractility during reperfusion (Supplementary Fig. 3). *Mif*$^{-/-}$ hearts subjected to *in vivo* left coronary occlusion/reperfusion showed 2.3-fold greater infarct size compared to wild-type controls (Fig. 3d). These results indicate that MIF promotes early adaptive responses in the heart during ischaemia-reperfusion.

Human gene mutations influence AMPK signalling and MIF expression. Individuals with rare mutations in the AMPK γ2 subunit

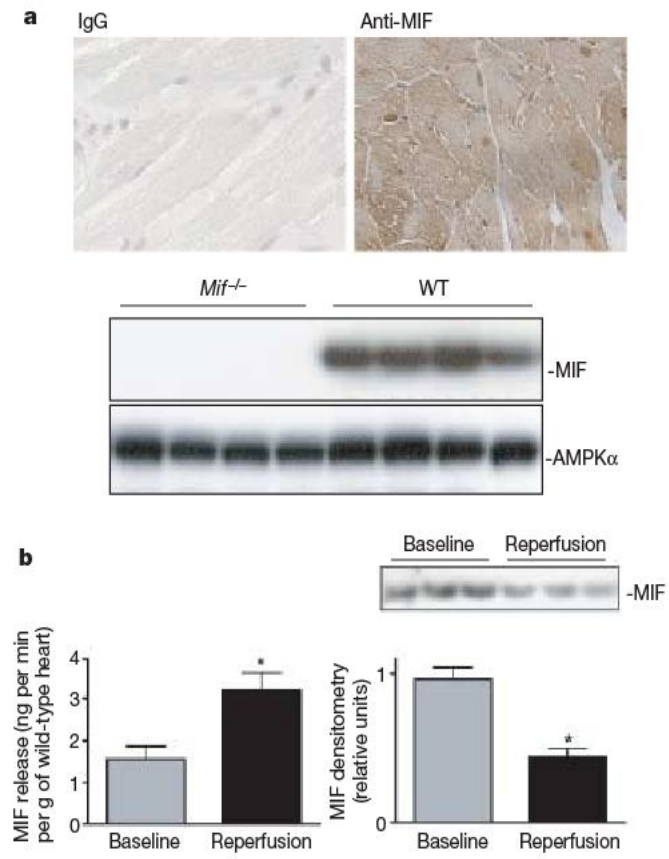

Figure 2 | Heart MIF expression and release triggered by ischaemia. **a**, Immunohistochemistry of wild-type (WT) mouse hearts with MIF antibody or non-immune immunoglobulin G (IgG). Immunoblots of heart lysates confirm the lack of immunoreactivity of the MIF antibody in *Mif*$^{-/-}$ hearts. Total AMPK is shown for loading comparison. **b**, Coronary effluent MIF production from wild-type hearts during baseline normal perfusion or during reperfusion after 10 min of ischaemia. MIF concentration was multiplied by the coronary flow to calculate the production rate. $*P = 0.01$, versus baseline by unpaired *t*-test comparing means of MIF concentration at five baseline and five reperfusion time points. MIF immunoblots of heart homogenates quantified by densitometry. $*P = 0.003$ versus control perfusions, $n = 2$–3 hearts each. Values are means ± s.e.m.

(PRKAG2, GeneID 51422) develop glycogen overload cardiomyopathy and Wolf–Parkinson–White syndrome[26]. A common polymorphism in the human *MIF* promoter, containing 5, 6, 7 or 8 CATT tetra-nucleotide repeat units (–794 $CATT_{5-8}$), also has functional consequences on *MIF* expression[5]. The $CATT_5$ allele demonstrates low *MIF* promoter activity compared to the others[5] and has been associated with less severe clinical manifestations of inflammatory diseases such as asthma[11], cystic fibrosis[27] and rheumatoid arthritis[5], presumably owing to decreased MIF signalling. The *MIF* promoter genotype varies in the population according to ethnicity, but the low expression genotype is relatively common with 6% of Caucasians and 14.5% of African-Americans homozygous for the –794 $CATT_5$ allele[28]. Despite demonstrable changes in *MIF* promoter activity, there are few data demonstrating the influence of the low expression genotype on the level of cellular MIF release.

Thus, we examined whether polymorphisms in the human *MIF* promoter might lead to functional differences in MIF secretion and cellular AMPK activation, using early passage human dermal fibroblasts. Cells from three of seven subjects were homozygous for the low expression −794 $CATT_5$ allele ('5/5' genotype) and the remainder had at least one high expression 6-, 7- or 8-CATT repeat allele ('non-5/5' genotype). The 5/5 cells had significantly less MIF release into the culture media, during both normal and hypoxic incubations, when compared to non-5/5 cells (Fig. 4a). Reduced MIF release from the 5/5 cells was associated with less AMPK phosphorylation during hypoxic stress (Fig. 4b). To determine whether the relative MIF deficiency in the 5/5 cells was responsible for the impaired AMPK activation during hypoxic stress, MIF (10 ng ml^{-1}) was added to the media during hypoxic incubation. Exogenous MIF restored hypoxic AMPK activation in the 5/5 cells to levels that were equivalent to the non-5/5 cells (Fig. 4b). In contrast, MIF did not augment AMPK activation in hypoxic non-5/5 fibroblasts (Fig. 4b). Similarly, the addition of exogenous MIF to hypoxic rat heart muscles did not augment AMPK activation (Supplementary Fig. 4). These data indicate that endogenous MIF release maximally modulates AMPK phosphorylation during hypoxia in normal heart tissue and cells. However, in relatively MIF-deficient cells (that is the 5/5 *MIF* promoter genotype), which have diminished MIF secretion during hypoxia, exogenous MIF augmented AMPK activation. The results indicate that recombinant MIF (or MIF agonists) might have a therapeutic effect by increasing AMPK activation during ischaemia or hypoxia in selected individuals with the low-expression 5/5 *MIF* promoter genotype. Thus, these experiments demonstrate that a common polymorphism in the *MIF* promoter leads to differential MIF release, which has consequences in cellular stress signalling in human cells. They also imply that exogenous MIF might have a beneficial effect in hypoxic tissues, specifically in patients with the 5/5 genotype.

Taken together with the results implicating MIF in the activation of AMPK in the ischaemic heart, these data raise the possibility that a common polymorphism in the *MIF* promoter influences the susceptibility of patients with coronary artery disease to ischaemic injury. AMPK is under current investigation as a potential target molecule for the treatment of type 2 diabetes, because of its metabolic actions that increase skeletal muscle glucose uptake and suppress hepatic glucose production. AMPK is also a potential target in ischaemic heart disease, because of its cardioprotective effects[1] and potential role in ischaemic preconditioning[29]. Treatment with MIF or MIF agonists warrants further study as an adjunctive therapy targeted at

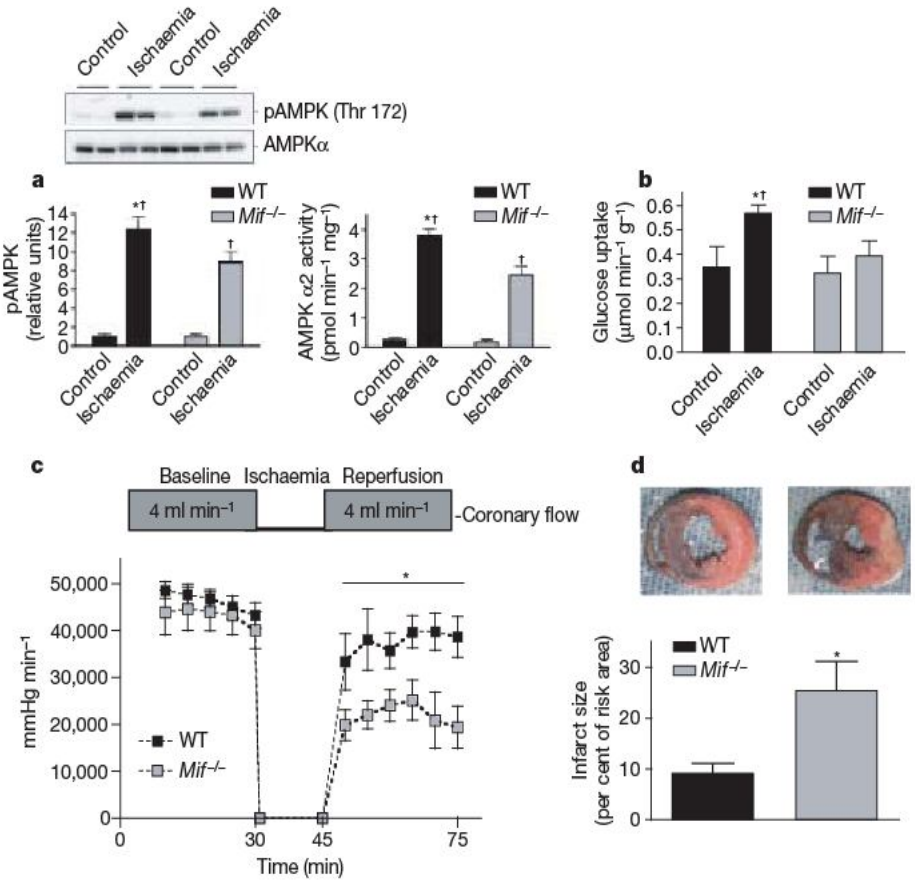

Figure 3 | Genetic MIF deletion impairs ischaemic heart AMPK activation and glucose uptake, and exacerbates post-ischaemic cardiac dysfunction and injury. **a**, AMPK phosphorylation and activity after ischaemic or control perfusions. $*P < 0.05$, versus $Mif^{-/-}$ ischaemic hearts; $\dagger P < 0.05$, ischaemic versus control, $n = 3$–4 hearts for each genotype. **b**, Glucose uptake during control perfusion and during reperfusion after ischaemia ($n = 5$ for each genotype). $*P = 0.01$, versus wild-type baseline, $\dagger P = 0.04$, versus $Mif^{-/-}$ reperfusion. **c**, Heart-rate–left-ventricular-developed pressure product during control perfusion and post-ischaemic reperfusion. $n = 6$–7 hearts for each genotype. $*P = 0.03$, by repeated measures ANOVA during reperfusion. **d**, Myocardial infarction induced by 15 min of left coronary occlusion *in vivo* followed by 4 h of reperfusion. Viable myocardium stained red with TTC; infarcted tissue, white; and normal non-ischaemic tissue, blue. The infarct area was quantified and expressed as a per cent of the ischaemic area at risk. $*P = 0.04$ versus wild type. $n = 5$–6 hearts per genotype. Values are means ± s.e.m.

AMPK activation during acute myocardial ischaemia or infarction. To the extent that MIF is released from the heart during ischaemic preconditioning, MIF agonists might also augment preconditioning by increasing AMPK activation during ischaemia. Therapy directed at AMPK might prove most effective in patients with low-expression *MIF* promoter polymorphisms. These hypotheses deserve further investigation and might also be addressed by analysis of gene banks from large cardiovascular clinical trials.

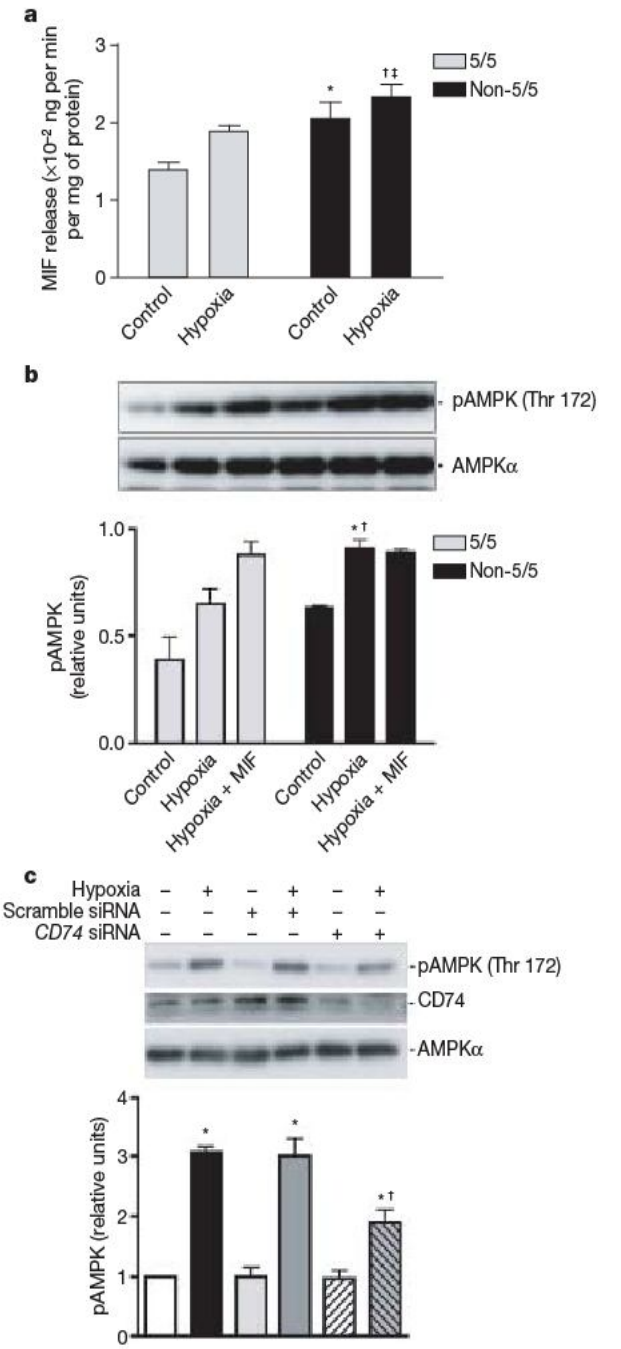

Figure 4 | Human *MIF* promoter genotype determines MIF secretion and AMPK activation during hypoxia. a, MIF secretion from human fibroblasts homozygous for the *MIF* promoter allele containing five CATT repeats (5/5 CATT, $n = 3$) or 6, 7 or 8 CATT repeat alleles (non-5/5 genotype, $n = 4$). $*P = 0.03$, versus 5/5 control cells, $\dagger P = 0.05$, versus 5/5 hypoxic cells, $\ddagger P = 0.03$ versus non-5/5 control cells. **b**, AMPK activation under control or hypoxic conditions with or without 10 ng ml^{-1} MIF. $*P = 0.01$, versus 5/5 and non-5/5 control cells; $\dagger P = 0.04$, versus 5/5 hypoxic cells. **c**, Human fibroblasts (non-5/5 genotype, $n = 4$) treated with MIF receptor *CD74* siRNA or control siRNA before hypoxia. Immunoblots show AMPK phosphorylation, CD74 and AMPK expression. The ratios of phosphorylated to total AMPK are expressed relative to control muscles. $*P = 0.01$, versus control; $\dagger P = 0.01$, versus control siRNA hypoxia. $n = 3$ experiments. Values are means ± s.e.m.

To define the proximal mechanisms linking MIF and AMPK activation better, we next examined whether components of MIF cell-surface receptor complex, which is comprised of the ligand-binding component, CD74 (ref. 14), and the signal-transducing component, CD44 (ref. 15), is involved in AMPK activation during hypoxia. Treatment of human fibroblasts with a CD74-specific short interfering RNA (siRNA) decreased MIF receptor CD74 protein expression and blunted hypoxia-stimulated AMPK phosphorylation (Fig. 4c). We also studied MIF-induced AMPK phosphorylation in $CD74^{null}/CD44^{null}$ COS-7/M6 cells that were stably transfected with either *CD74* alone, *CD44* alone, or *CD74* together with *CD44* (ref. 15). COS-7/M6 cells that expressed *CD74* or *CD44* alone showed no AMPK response to either hypoxia or exogenously added MIF. In contrast, COS-7/M6 cells expressing both transmembrane proteins showed significant AMPK phosphorylation responses (Supplementary Fig. 5). These results support an important role for the two-component receptor complex, consisting of the MIF binding CD74 protein and the signal-transducing CD44 protein, in MIF-mediated AMPK signalling during cellular hypoxia in the heart. A CD74-dependent interaction between MIF and CXCR2 also has been reported and has a role in inflammatory cell recruitment[10]. Whether MIF activation of CXCR2 also has a role in the cellular response to hypoxic injury beyond its migratory function is worthy of additional investigation.

In conclusion, these results define new models of both MIF action and AMPK activation, establishing a link between pathways central to inflammation and metabolism. MIF release leads to autocrine/paracrine activation of the AMPK-signalling pathway in the ischaemic heart. In other inflammatory disease states, high levels of MIF signalling, potentially activating additional pathways, might be deleterious. A common polymorphism in the human *MIF* promoter influences AMPK activation, and might predispose susceptible individuals to ischaemic injury and provide a potential new risk marker for patients with coronary artery disease.

METHODS SUMMARY

Rat heart muscles were incubated *in vitro*[21] to assess the effects of exogenous MIF on AMPK activation and downstream signalling, as well as to determine the role of endogenous MIF on the AMPK pathway during hypoxia. Isolated hearts from wild-type or $Mif^{-/-}$ mice were retrogradely perfused *in vitro*[1], as well as subjected to *in vivo* coronary occlusion/reperfusion, to examine the role of endogenous MIF in AMPK activation and myocardial injury during ischaemia. Human fibroblasts with various polymorphisms in the *MIF* promoter were incubated under hypoxic conditions to determine the role of *MIF* genotype on hypoxic AMPK activation, as well as the mechanism of AMPK activation by siRNA knockdown of the CD74 receptor. CD74 and/or CD44 were expressed in COS cells to assess the role of these receptors in the MIF response.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Information The authors declare competing financial interests: details accompany the HTML version of this paper at www.nature.com/nature. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to L.H.Y. (lawrence.young@yale.edu).

LETTERS

DBC1 is a negative regulator of SIRT1

Ja-Eun Kim[1], Junjie Chen[1] & Zhenkun Lou[2]

The NAD-dependent protein deacetylase Sir2 (silent information regulator 2) regulates lifespan in several organisms[1–3]. SIRT1, the mammalian orthologue of yeast Sir2, participates in various cellular functions[4–7] and possibly tumorigenesis[8]. Whereas the cellular functions of SIRT1 have been extensively investigated, less is known about the regulation of SIRT1 activity. Here we show that Deleted in Breast Cancer-1 (DBC1), initially cloned from a region (8p21) homozygously deleted in breast cancers[9], forms a stable complex with SIRT1. DBC1 directly interacts with SIRT1 and inhibits SIRT1 activity *in vitro* and *in vivo*. Downregulation of DBC1 expression potentiates SIRT1-dependent inhibition of apoptosis induced by genotoxic stress. Our results shed new light on the regulation of SIRT1 and have important implications in understanding the molecular mechanism of ageing and cancer.

SIRT1 is the mammalian orthologue of yeast Sir2, which has emerged as an important regulator of ageing[10,11]. SIRT1 deacetylates diverse substrates including PGC-1α (ref. 4), p53 (refs 5, 6), forkhead transcription factor (FOXO)[7,12–14], NF-κB[15], Ku70 (ref. 16), MyoD[17] and histones[18]. Thus it influences gene silencing, apoptosis, stress resistance, senescence, and fat and glucose metabolism. The combination of these cellular functions might contribute to an anti-ageing effect in mammals although SIRT1 could also limit replicative lifespan after chronic genotoxic stress[19]. On the other hand, SIRT1 activity has also been linked to tumorigenesis. SIRT1 is important for tumour cell growth and survival, possibly due to SIRT1's anti-apoptotic effect[20–22]. In addition, SIRT1 participates in the silencing of tumour suppressor genes[23] and SIRT1 overexpression has been observed in cancer cells[8,15,24,25]. Therefore, SIRT1 could have important implications in both ageing and cancer. It is conceivable that SIRT1 activity might be a 'double-edged sword' that requires tight regulation. Despite the extensive studies of SIRT1 function, the regulation of SIRT1 is less understood.

To elucidate potential regulators of SIRT1, large-scale affinity purification using SIRT1-stable cell lines was performed (Fig. 1a and Supplementary Fig. 1a). We reproducibly found DBC1, also called p30 DBC, as a major SIRT1-associated protein. Some previously identified SIRT1-associated proteins (for example HIC1 or E2F1) or SIRT1 substrates (for example p53 or FOXO) were not found in this purification, probably because of the condition we used, which might have led to dissociation of these proteins during purification. In addition, purification of endogenous SIRT1 also identified DBC1 as a major SIRT1-associated protein (Fig. 1b). DBC1 messenger RNA is lost in several breast, lung and colon cancer cell lines[9]. A previous study suggested that DBC1 may play a role in tumour-necrosis factor (TNF-α)-mediated apoptosis[26]. However, it is not yet clear whether DBC1 is a tumour suppressor and how it functions in the cell.

A substantial portion of endogenous DBC1 and SIRT1 co-immunoprecipitated together (Fig. 1c), suggesting a strong DBC1–SIRT1 interaction. DBC1 could not be detected in anti-SIRT1 immunoprecipitates from SIRT1-knockdown cells and vice versa (Supplementary Fig. 1b), supporting the specificity of this interaction. In addition, only SIRT1, but not SIRT6 or SIRT7 (other nuclear sirtuins), interacts with endogenous DBC1 (Fig. 1d). Affinity purification of DBC1 identified SIRT1, but not any of SIRT2–7, as a major DBC1-associated protein (data not shown). Furthermore, we found that DBC1 interacted specifically with SIRT1 in insect cells infected with baculovirus expressing each recombinant protein, suggesting a direct interaction between DBC1 and SIRT1 (Fig. 1e).

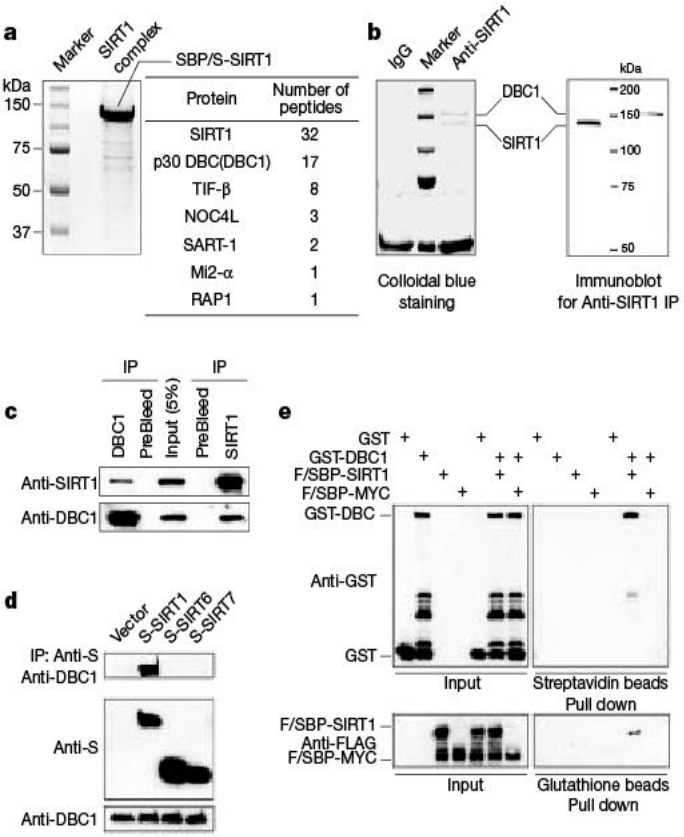

Figure 1 | DBC1 interacts with SIRT1 *in vivo*. **a**, The pull-down products from tandem affinity purification using 293T cells stably expressing tagged SIRT1 were separated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and visualized by Colloidal blue staining. The data from mass spectrometry analysis are shown in a table on the right. **b**, Purification of endogenous SIRT1 was performed in 293T cells with anti-SIRT1 antibody and visualized by Colloidal blue staining. The identities of DBC1 and SIRT1 were confirmed by mass spectrometry (data not shown) and immunoblotting. **c**, Endogenous association between DBC1 and SIRT1 in A549 cells was performed by co-immunoprecipitation experiments. **d**, Constructs encoding S-tagged SIRT1, 6 and 7 were transfected into 293T cells. **e**, Sf9 cells were infected with baculoviruses encoding GST or GST-DBC1 together with those encoding F(FLAG)/SBP-SIRT1 or F/SBP-Myc. **d**, **e**, Immunoprecipitation (or pull-down) and immunoblotting were performed 48 h post-transfection or post-infection as indicated.

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To identify the regions of DBC1 that are responsible for the DBC1–SIRT1 interaction, we generated deletion mutants of DBC1 (Fig. 2a and Supplementary Fig. 2a). Deletion of DBC1 residues 1–264 (Δ1–264) abolished the binding of DBC1 with SIRT1. Further deletion analysis allowed us to map the SIRT1-binding region of DBC1 to residues 243–264, which contains a leucine zipper (LZ) motif (Fig. 2a). Indeed, SIRT1 formed a stable complex with full-length DBC1 in insect cells, but not with DBC1 with the leucine zipper motif deleted (Supplementary Fig. 2b), suggesting that the DBC1 LZ motif is required for its interaction with SIRT1.

Similarly, we generated deletion mutants of SIRT1 (Fig. 2b). The DBC1-binding region of SIRT1 was mapped to the SIRT1 catalytic domain. A direct interaction between the LZ motif of DBC1 and the catalytic domain (cat) of SIRT1 was confirmed by infection of insect cells with baculovirus encoding each protein (Fig. 2c). Furthermore, point mutation at the catalytic domain (H363Y) of SIRT1 greatly diminished the DBC1–SIRT1 interaction (Supplementary Fig. 2c). Collectively, these findings indicate that the DBC1 LZ motif and the SIRT1 catalytic domain are both necessary and sufficient for the interaction between DBC1 and SIRT1.

Because SIRT1 is a protein deacetylase and DBC1 binds the SIRT1 catalytic domain, we investigated whether DBC1 would regulate the deacetylase activity of SIRT1. We used p53 acetylated by p300 (ref. 27), a well-studied SIRT1 substrate, to measure SIRT1 activity *in vitro* in the absence or presence of DBC1. As expected, SIRT1 deacetylated p53, and the addition of suramin, a SIRT1 inhibitor, strongly inhibited p53 deacetylation by SIRT1 (Fig. 3a). Interestingly, DBC1 blocked the ability of SIRT1 to deacetylate p53 (Fig. 3a and Supplementary Fig. 3c, d). These results suggest that DBC1 negatively regulates SIRT1 activity.

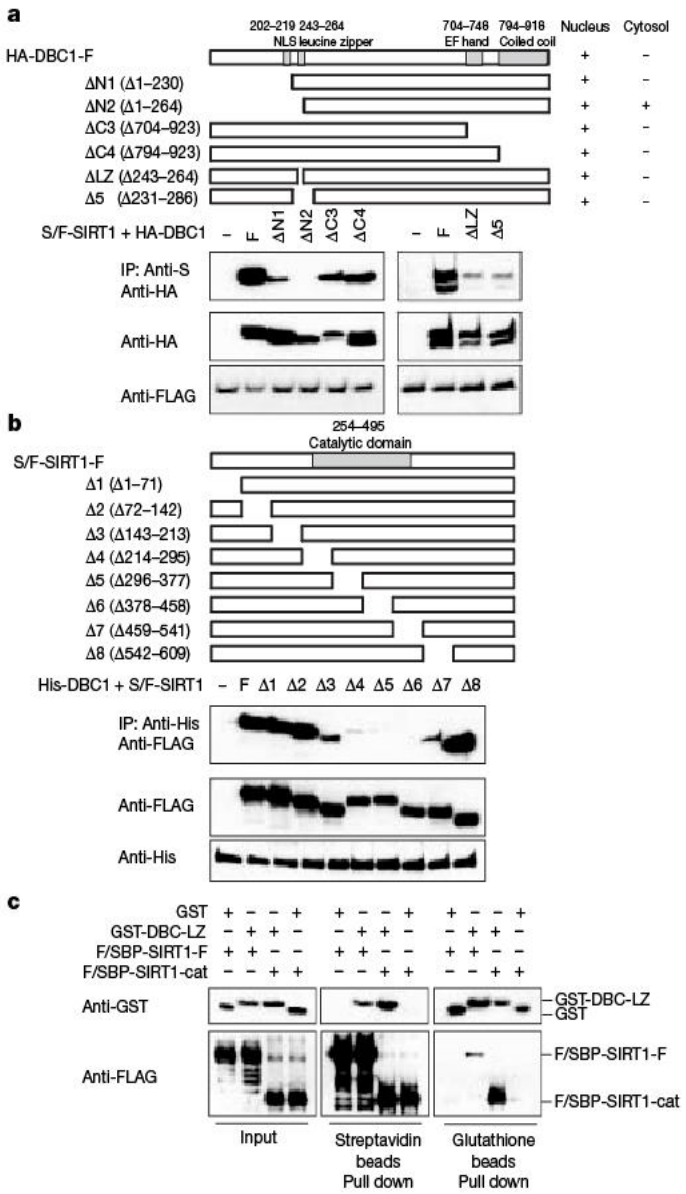

Figure 2 | The leucine zipper motif of DBC1 and the catalytic domain of SIRT1 are required for the DBC1–SIRT1 interaction. **a**, Plasmids encoding HA-tagged full-length or deletion mutants of DBC1 were co-transfected with plasmids encoding S/F(FLAG)-tagged full-length SIRT1 into 293T cells. **b**, Plasmids encoding S/F-tagged full-length or deletion mutants of SIRT1 were co-transfected with plasmids encoding His-tagged full-length DBC1 in 293T cells. **c**, Sf9 cells were co-infected with baculoviruses expressing GST or GST-DBC1 leucine zipper (LZ, residues 243–264) and F/SBP-SIRT1-full-length or SIRT1-catalytic region (cat, residues 143–541). **a–c**, Immunoprecipitation (or pull-down) and immunoblotting were performed 48 h post-transfection or post-infection as indicated.

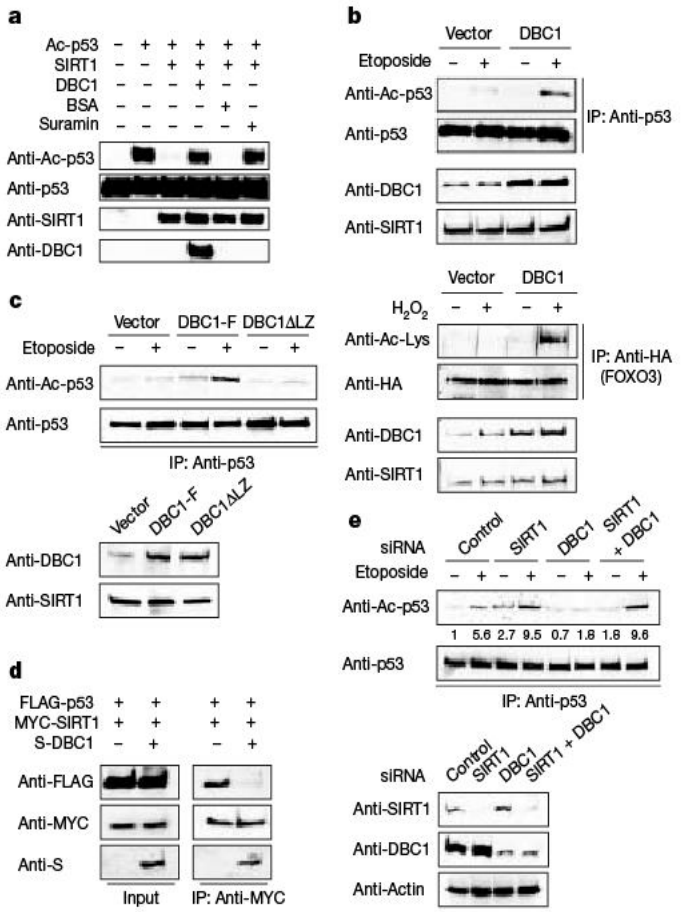

Figure 3 | DBC1 inhibits SIRT1 deacetylase activity *in vitro* and *in vivo*. **a**, Acetylated p53 was incubated with either SIRT1 or the SIRT1–DBC1 complex in the presence of NAD as indicated. p53 acetylation was then assessed by immunoblotting with anti-acetyl-p53 antibody. **b**, A549 cells were transfected with vector control or plasmid encoding DBC1. Forty-eight hours later, cells were treated with MG132 (50 μM, 20 min) to stabilize the total level of p53 followed by etoposide treatment (20 μM, 1 h). p53 acetylation was assessed by immunoprecipitation of p53 followed by immunoblotting with anti-acetyl-p53 antibody. To detect the acetylation of FOXO3, HA-tagged FOXO3 was co-transfected with vector control or a construct encoding DBC1 in 293T cells. Forty-eight hours post-transfection, cells were treated with hydrogen peroxide (0.5 mM, 1 h) and the acetylation of FOXO3 was detected as indicated. **c**, A549 cells were transfected with plasmids encoding full-length DBC1 (DBC-F) or DBC1 with the LZ motif deleted (DBCΔLZ). p53 acetylation was evaluated as described in **b**. **d**, Plasmids encoding FLAG-p53 and MYC-SIRT1 with or without plasmids encoding S-DBC1 were co-transfected in 293T cells. The binding of p53 to SIRT1 was detected by immunoprecipitation followed by immunoblotting as indicated. **e**, A549 cells were transfected with control, SIRT1, DBC1, or SIRT1 and DBC1 siRNAs. Seventy-two hours after siRNA transfection, cells were treated and p53 acetylation was then examined as described in **b**. Ac-p53 level was quantified and normalized by total p53 using densitometry.

To explore the negative regulation of SIRT1 by DBC1 *in vivo* further, we examined whether the deacetylation of SIRT1 substrates such as p53 and FOXO3 would be inhibited when DBC1 is overexpressed. DBC1 overexpression resulted in hyperacetylation of p53 in response to etoposide (VP-16) treatment, whereas SIRT1 expression was not affected by the overexpression of DBC1 (Fig. 3b). Likewise, overexpression of DBC1 enhanced the acetylation of FOXO3 in response to hydrogen peroxide (Fig. 3b). These results suggest that overexpression of DBC1 inhibits SIRT1 activity *in vivo*.

It is possible that the binding of DBC1 to the catalytic domain of SIRT1 inhibits SIRT1 activity. If so, a DBC1 mutant that does not bind SIRT1 should not affect SIRT1 activity and the acetylation status of SIRT1 substrates. In contrast to DBC1-F, overexpression of DBC1ΔLZ failed to increase p53 acetylation (Fig. 3c and Supplementary Fig. 4a). These results suggest that DBC1 inhibits SIRT1 activity through a direct interaction between DBC1 and SIRT1.

Given that DBC1 binds to the SIRT1 catalytic domain, it is also possible that DBC1 can block the association between SIRT1 and its substrates, thereby inhibiting SIRT1 activity. Indeed, although SIRT1 bound to its substrate p53 or FOXO, this binding was significantly reduced when DBC1 was co-expressed in these cells (Fig. 3d and Supplementary Fig. 4b), suggesting that at least one mechanism of the inhibitory function of DBC1 is to block substrate access.

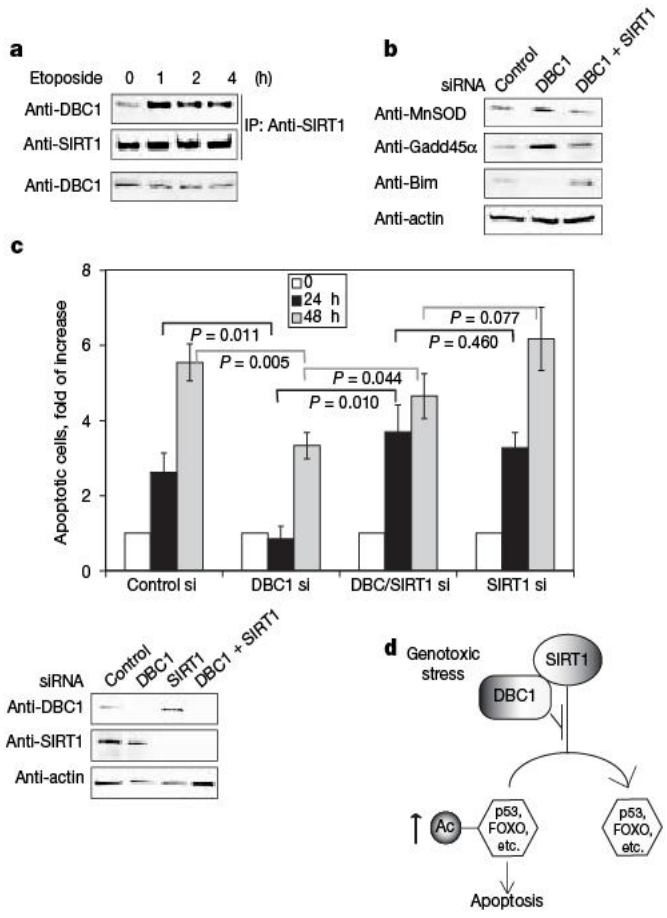

Figure 4 | DBC1 regulates SIRT1 function after stress stimuli. a, A549 cells were treated with etoposide for 1, 2 or 4 h. **b**, **c**, A549 cells were transfected with control, *DBC1*, or *DBC1* and *SIRT1* siRNAs and lysed 72 h after transfection. **a**–**c**, Cell lysates were analysed by immunoprecipitation or immunoblotting as indicated. **c**, Seventy-two hours later, siRNA-transfected cells were treated with etoposide (20 μM, 24 and 48 h). The apoptotic cells were determined by Annexin V staining. The *y* axis represents fold of increase of apoptotic cells compared with mock-treated sample. Data are presented as mean ± s.e.m.; $n = 3$, *P* value was determined by Student's *t*-test. **d**, The model shows that DBC1 acts as a SIRT1 inhibitor and regulates SIRT1-dependent functions.

To confirm further the inhibitory role of DBC1 on SIRT1 *in vivo*, we downregulated DBC1 expression by short interfering RNA (siRNA). As predicted, downregulation of DBC1 resulted in hypoacetylation of p53 in response to either etoposide or hydrogen peroxide (Fig. 3e and Supplementary Fig. 4c). Similar results were obtained when another *DBC1* siRNA was used (Supplementary Fig. 4d). To verify that the hypoacetylation of p53 in *DBC1* siRNA-transfected cells is due to the hyperactivation of SIRT1, cells were transfected with *DBC1* siRNA together with *SIRT1* siRNA. Co-transfection of these siRNAs reversed the hypoacetylation induced by *DBC1* siRNA (Fig. 3e), strongly supporting the role of endogenous DBC1 in inhibiting SIRT1 activity in the cell.

These results led us to further study whether DBC1 regulates SIRT1-dependent functions. In response to genotoxic stress, SIRT1 inhibits apoptosis and promotes cell survival through several pathways such as p53 (refs 5, 6), Ku70 (ref. 16) and E2F1 (ref. 28). In addition, SIRT1 also attenuates apoptosis by deacetylation of FOXO transcription factors, which leads to the transcriptional activation of stress resistance genes *MnSOD* and *Gadd45* and the transcriptional repression of proapoptotic target Bim[7,12–14]. Interestingly, we observed that the binding of DBC1 to SIRT1 increased after etoposide treatment (Fig. 4a), suggesting that DBC1 may inhibit SIRT1 activity at the early stage of stress response and therefore result in the observed hyperacetylation of SIRT1 substrates after stress stimuli. To investigate whether DBC1 affects SIRT1-dependent functions, we first examined the expression level of FOXO target genes. Downregulation of DBC1 increased the expression of MnSOD and Gadd45α (Fig. 4b). Conversely, downregulation of DBC1 decreased Bim expression (Fig. 4b). Similar results were obtained using a second DBC1 siRNA (data not shown). These results are consistent with an enhanced SIRT1 activity after DBC1 depletion. To confirm that these changes in gene expression are due to the hyperactivation of SIRT1, cells were co-transfected with *DBC1* siRNA and *SIRT1* siRNA. Downregulation of SIRT1 together with DBC1 reversed the gene expression changes caused by downregulation of DBC1 alone (Fig. 4b). These results suggest that DBC1 negatively regulates SIRT1-dependent gene expression and would predict that downregulation of DBC1 will render cells resistant to cellular stress. Indeed, cells transfected with *DBC1* siRNA underwent less apoptosis than those transfected with control siRNA after treatment with etoposide or hydrogen peroxide. Co-transfection of *DBC1* siRNA with *SIRT1* siRNA reversed the anti-apoptotic effect of DBC1 siRNA (Fig. 4c and Supplementary Fig. 5a). These results indicate that DBC1 regulates SIRT1-dependent stress responses.

Several studies suggest that SIRT1 may be regulated at the transcriptional level by FOXO3, p53, HIC1 or E2F1 (refs 25, 28, 29). SIRT1 activity may also be controlled by the NAD level[30]. Our work demonstrates that SIRT1 activity can be regulated through a direct protein–protein interaction. DBC1 forms a stable complex with SIRT1 and negatively regulates SIRT1 activity. This inhibition is possibly due to a blockage of substrate access because DBC1 binds directly to the SIRT1 catalytic domain. Structural analysis is needed to further elucidate the mechanism by which DBC1 inhibits SIRT1 activity. The inhibitory function of DBC1 on SIRT1 could augment cell death under stress stimuli. In this regard, DBC1 may act as a tumour suppressor, and loss of DBC1 would result in the inhibition of cell death and promote tumorigenesis. Future studies using DBC1 knockout mice will test directly whether DBC1 is a bona fide tumour suppressor *in vivo*. Given the complex connection between ageing and cancer, our findings will have significant impact on understanding of the molecular pathways that regulate ageing and cancer progression.

METHODS SUMMARY

293T cells stably expressing streptavidin-binding peptide (SBP)- and S-tagged SIRT1 were used for two-step affinity purification to purify the SIRT1 complex. Co-immunoprecipitation was used to detect a protein–protein interaction.

Transient transfection of siRNA was used to decrease the level of specific proteins. *In vitro* SIRT1 deacetylation assay was performed with acetylated p53 as a substrate. The level of apoptosis was determined by Annexin V staining.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions J.K. and Z.L. performed the experimental work and data analysis. J.K., J.C. and Z.L. wrote the paper. All authors discussed the results and commented on the manuscript.

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LETTERS

Negative regulation of the deacetylase SIRT1 by DBC1

Wenhui Zhao[1]*, Jan-Philipp Kruse[1]*, Yi Tang[1]*, Sung Yun Jung[2], Jun Qin[2] & Wei Gu[1]

SIRT1 is an NAD-dependent deacetylase critically involved in stress responses, cellular metabolism and, possibly, ageing[1–15]. The tumour suppressor p53 represents the first non-histone substrate functionally regulated by acetylation and deacetylation[16,17]; we and others previously found that SIRT1 promotes cell survival by deacetylating p53 (refs 4–6). These results were further supported by the fact that p53 hyperacetylation and increased radiation-induced apoptosis were observed in Sirt1-deficient mice[10]. Nevertheless, SIRT1-mediated deacetylase function is also implicated in p53-independent pathways under different cellular contexts, and its effects on transcriptional factors such as members of the FOXO family and PGC-1α directly modulate metabolic responses[1–15]. These studies validate the importance of the deacetylase activity of SIRT1, but how SIRT1 activity is regulated *in vivo* is not well understood. Here we show that DBC1 (deleted in breast cancer 1) acts as a native inhibitor of SIRT1 in human cells. DBC1-mediated repression of SIRT1 leads to increasing levels of p53 acetylation and upregulation of p53-mediated function. In contrast, depletion of endogenous *DBC1* by RNA interference (RNAi) stimulates SIRT1-mediated deacetylation of p53 and inhibits p53-dependent apoptosis. Notably, these effects can be reversed in cells by concomitant knockdown of endogenous *SIRT1*. Our study demonstrates that DBC1 promotes p53-mediated apoptosis through specific inhibition of SIRT1.

To understand the regulation of SIRT1-mediated deacetylation *in vivo*, biochemical purification was used to identify cellular factors that stably interact with SIRT1. We isolated physiologically formed protein complexes containing SIRT1 from cell extracts of native HeLa cells by conducting affinity chromatography with affinity-purified antisera raised against the carboxy (C) terminus (amino acids 480–737) of SIRT1 (Supplementary Fig. 1a). As expected, we identified SIRT1 as the major component of the complexes, but several protein bands were also co-purified with SIRT1. Mass spectrometry of a prominent protein band of approximately 130 kilodaltons (kDa) from the SIRT1 complexes revealed peptide sequences corresponding to the DBC1 protein (Supplementary Fig. 1b, Gi: 24432106). The *DBC1* gene was initially identified as it is localized to a region of chromosome 8p21 that was homozygously deleted in human breast cancer; however, the molecular function of DBC1 is poorly understood[18,19].

To examine the interaction between endogenous DBC1 and SIRT1, cell extracts from human osteosarcoma U2OS cells were immunoprecipitated with the anti-SIRT1 antibody or with the control IgG. As expected, western blot analysis revealed that DBC1 was clearly detected in the immunoprecipitations obtained with the anti-SIRT1 antiserum (lane 3, Fig. 1a) but not with the control antibody (lane 2). Previous studies indicate that HIC1 can also interact with SIRT1 (ref. 11); nevertheless, we failed to detect a strong interaction between HIC1 and SIRT1 in these cells under the same conditions. To prove the specificity of the SIRT1 antibody, we performed the co-immunoprecipitation in SIRT1-depleted U2OS cells treated with

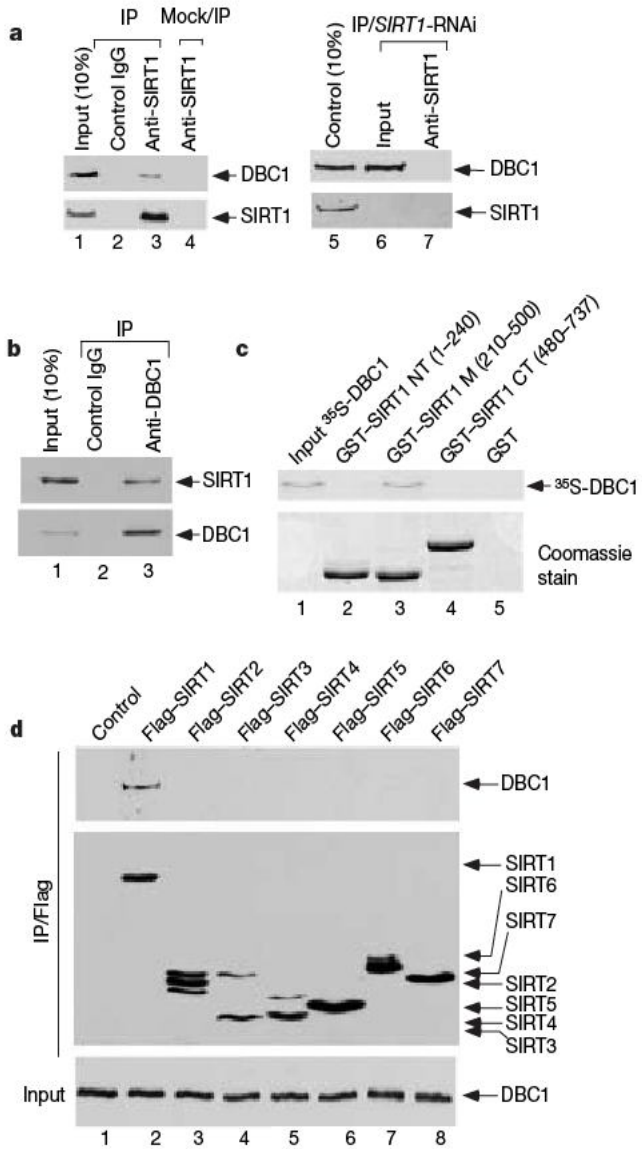

Figure 1 | DBC1 interacts with SIRT1 *in vivo* and *in vitro*. **a**, Co-immunoprecipitation of DBC1 with SIRT1. Western blot analysis of U2OS whole-cell extracts and SIRT1-antibody, IgG and mock IP (lane 4) immunoprecipitates by DBC1 and SIRT1-antibody (left panel), and after *SIRT1* RNAi knockdown (right panel). **b**, Co-immunoprecipitation of SIRT1 by DBC1 from U2OS whole-cell extracts with DBC1 or IgG analysed by western blot. **c**, Direct interaction of DBC1 with GST–SIRT1-core (m) domain in *in vitro* GST pull-down assay detected by autoradiography. **d**, DBC1 interaction with Sirtuin family *in vivo*. 293 cells were transfected with Flag–SIRT1–7, and extracts (bottom panel) and M2-immunoprecipitates (upper panels) were analysed by western blot.

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SIRT1-specific RNAi (lanes 6 and 7). Indeed, we failed to detect any DBC1 from the anti-SIRT1 immunoprecipitates with these SIRT1-depleted cells (lane 7). We also performed a reciprocal co-immunoprecipitation assay. As shown in Fig. 1b, endogenous SIRT1 was readily immunoprecipitated with the DBC1-specific antibody (lane 3), but not with a control antibody (lane 2).

Next, we tested whether SIRT1 binds DBC1 *in vitro*. As shown in Fig. 1c, ^{35}S-labelled *in-vitro*-translated DBC1 bound the central core domain of SIRT1 (lane 3) but showed no affinity for either its amino (N)-terminal (lane 2) or C-terminal (lane 4) domains. Conversely, we identified the N terminus of DBC1 as the SIRT1-binding domain (Supplementary Fig. 3). Because the enzymatic core sequence represents the most conserved region within the mammalian SIRT protein family, we examined whether DBC1 interacts with other members of this family. Thus, Flag-tagged derivatives of the seven human SIRT polypeptides (SIRT1–7) were each expressed in 293 cells and extracts of the transfected cells were immunoprecipitated with the anti-Flag antibody. Western blots revealed that endogenous DBC1 was clearly detected in the immunoprecipitates of Flag–SIRT1 (lane 2, Fig. 1d). Although similar expression levels for all seven Flag–SIRT polypeptides were detected, none of the other SIRT proteins (SIRT2–7) were able to co-immunoprecipitate DBC1 (lanes 3–8, Fig. 1d). These results demonstrate the specificity of the SIRT1 and DBC1 interaction.

Interestingly, when purified Flag–SIRT1 complexes from human cells were analysed by gel-filtration chromatography on a Superose 12 column, we observed that SIRT1 and DBC1 polypeptides co-eluted in fraction 15 with an apparent molecular mass of 440 kDa (lane 4, Fig. 2a). In contrast, a DBC1-free form of SIRT1 eluted in fractions 19–21 (lanes 6 and 7), suggesting that at least two distinct SIRT1 complexes exist in human cells. As expected, we found that SIRT1 from fraction 21 had a strong NAD-dependent deacetylase activity for p53 (lane 3, Fig. 2b). Surprisingly, however, no activity was detected with fraction 15 (lane 2), raising the notion that SIRT1-mediated deacetylation is inhibited by additional factors in the complexes, such as DBC1. To evaluate a role for DBC1 in regulating SIRT1 function, we examined whether DBC1 can inhibit the deacetylase activity of SIRT1 in a purified system. To this end, Flag-tagged forms of SIRT1 and DBC1 were purified under high-stringency conditions for *in vitro* deacetylation assays. As indicated in Fig. 2c, deacetylation of p53 was observed when the Flag–SIRT1 protein was incubated with acetylated p53 (lane 2). However, this activity was strongly repressed by Flag–DBC1 in a dose-dependent manner (lanes 3 and 4). DBC-mediated repression is apparently as potent as the

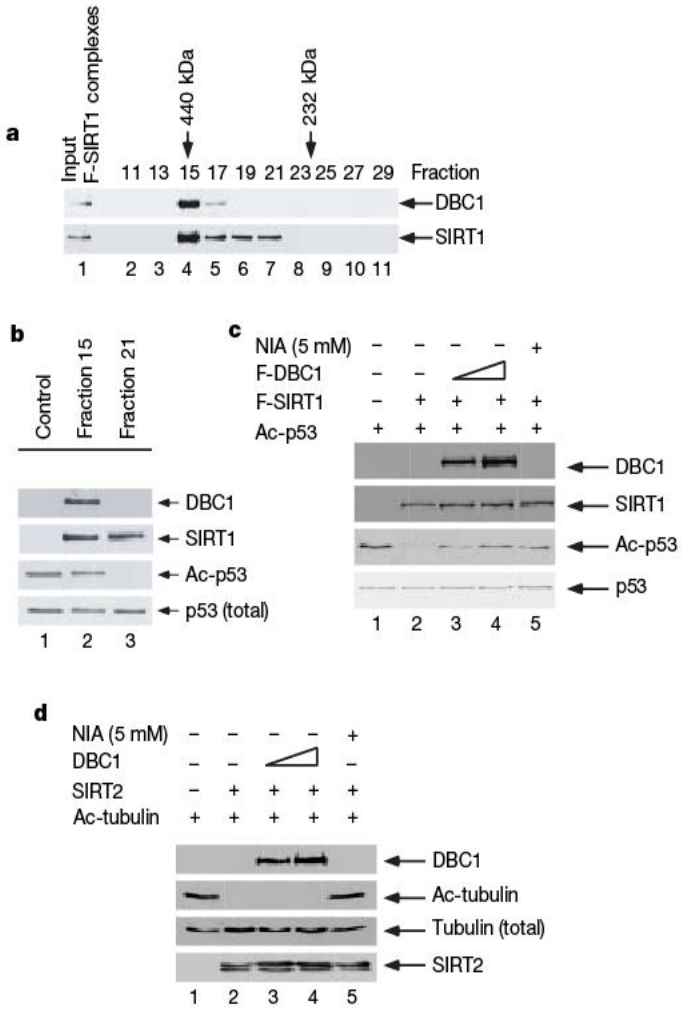

Figure 2 | DBC1 inhibits SIRT1-mediated deacetylation of p53. **a**, M2 purified Flag-SIRT1 (F-SIRT1) complexes from 293 cells were fractioned by size-exclusion chromatography and analysed by western blot as indicated. **b**, *In vitro* p53 deacetylation by SIRT1 fractions with or without DBC1. **c**, DBC1 inhibition of SIRT1-mediated deacetylation is dose dependent. Increasing amounts of purified F-DBC1 (lanes 2–4) or nicotinamide (lane 5) can inhibit highly purified F-SIRT1-mediated p53 deacetylation. **d**, DBC1 does not inhibit SIRT2-mediated tubulin deacetylation *in vitro*. Increasing amounts of purified F-DBC1 (lanes 2–4) do not inhibit purified F- SIRT2-mediated tubulin deacetylation. Deacetylation assays were analysed by western blot with the indicated antibodies.

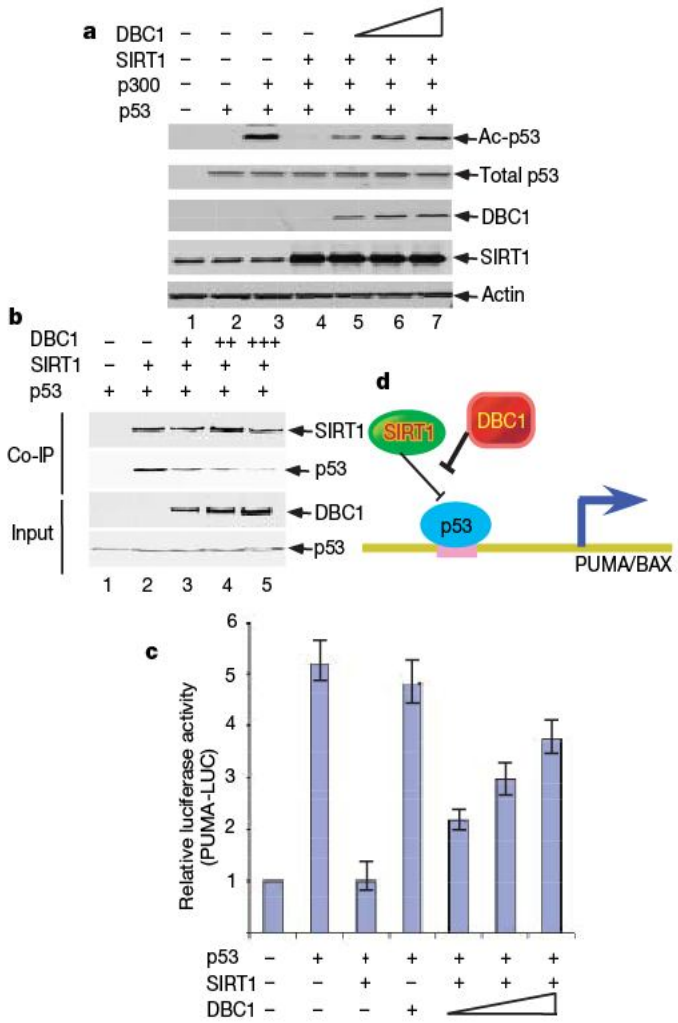

Figure 3 | DBC1 acts as an inhibitor of SIRT1 in human cells. **a**, DBC1 represses SIRT1 deacetylation activity *in vivo*. Transfected H1299 cell extracts were analysed by western blot using antibodies as shown. **b**, DBC1 inhibits SIRT1-mediated co-immunoprecipitation of p53 *in vivo*. H1299 cells were transfected as indicated and Flag–SIRT1 immunoprecipitations were analysed by western blot as shown. **c**, DBC1 expression rescues SIRT1-mediated repression of p53 transcriptional activation. H1299 cells were transfected with the PUMA-luciferase promoter construct and p53, SIRT1 and DBC1 as indicated. Lysates were assayed for the dual-luciferase activity. Error bars represent s.d., $n = 3$. **d**, Model showing DBC1 acting as an inhibitor of SIRT1-mediated repression of p53.

effects obtained with 5 mM of nicotinamide (NIA) (lane 5), a known inhibitor of SIRT1-mediated deacetylation[4].

Moreover, to prove the specificity of DBC-mediated inhibition of SIRT1 deacetylase activity, we examined the effect of DBC1 on SIRT2-mediated deacetylation of tubulin. As shown in Fig. 2d, deacetylation of tubulin was observed when the purified SIRT2 protein was incubated with acetylated tubulin, as previously reported[20]. This activity was also inhibited by nicotinamide (lane 5); however, tubulin deacetylation by SIRT2 was not affected by purified DBC1 polypeptides (lanes 3 and 4). Finally, p53 could also be deacetylated by purified HDAC1 complexes, as we have previously shown[17] (lane 2, Supplementary Fig. 4); nevertheless, this deacetylase activity was not repressed by DBC1 (lanes 3 and 4). Thus, these data demonstrate that DBC1-mediated inhibitory effects specifically act on SIRT1 deacetylase activity.

We then tested whether DBC1 expression rescues p53 from SIRT1-mediated deacetylation and repression in human cells. As expected, co-expression of SIRT1 induced p53 deacetylation (lane 4, Fig. 3a); however, the steady-state levels of acetylated p53 were restored by DBC1 expression in a dose-dependent manner (lanes 5–7). To elucidate the mechanism of DBC-mediated effects on SIRT1, we conducted a co-immunoprecipitation assay to test whether the interaction between SIRT1 and p53 is regulated by DBC1. As shown in Fig. 3b, p53 was co-immunoprecipitated with SIRT1 (lane 2). Notably, the p53–SIRT1 interaction was significantly abrogated by DBC1 expression in a dose-dependent fashion (lanes 3–5). These results suggest that DBC1 represses SIRT1 activity in human cells and that these effects may act, in part, through blocking the interactions between SIRT1 and substrates (p53).

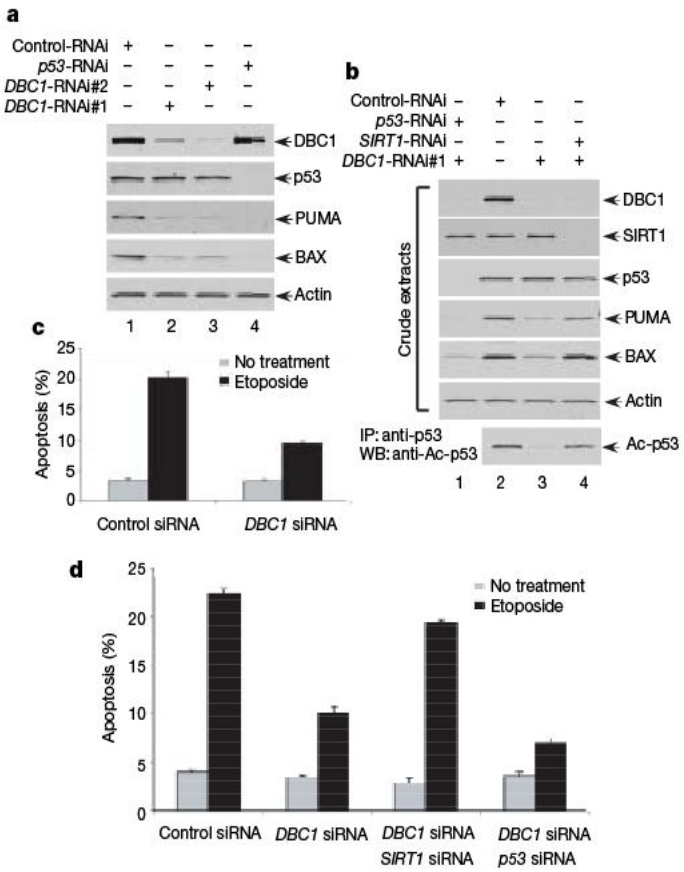

Figure 4 | siRNA-mediated knockdown of *DBC1* reduces p53 acetylation and its transcriptional and apoptotic activities. **a**, siRNA-mediated *DBC1* knockdown inhibits p53 proapoptotic target gene expression. Western blot of U2OS whole-cell extracts treated with siRNA as shown. **b**, *DBC1* siRNA effects on p53-dependent PUMA and BAX expression require SIRT1. Western blot of siRNA-treated U2OS cell extracts. p53 acetylation levels are shown after p53 immunoprecipitation. **c**, **d**, *DBC1* siRNA inhibits p53-mediated apoptosis after DNA damage. U2OS cells were treated with siRNA as indicated and treated with Etoposide (30 h) before assaying apoptosis by TUNEL staining (**c**, Supplementary Fig. 11) or Annexin V staining (**d**, Supplementary Fig. 12). Error bars represent s.d., $n = 3$ (**c**, **d**).

To explore the functional consequences of these interactions further, we tested whether DBC1 can influence SIRT1-mediated repression of p53 transcriptional activation. As shown in Fig. 3c, SIRT1 strongly suppressed p53-mediated transactivation of the PUMA reporter in a luciferase assay. Again, this SIRT1-mediated suppression was abrogated by DBC1 expression in a dose-dependent manner. These data indicate that DBC1 can enhance p53-dependent transactivation of PUMA by inhibiting SIRT1. Because homozygous deletion of the *DBC1* gene was reported in human cancers[18,21], inactivation of *DBC1* may enhance the deacetylase activity of SIRT1 and thereby lead to inhibition of p53 function (Fig. 3d).

To test the above hypothesis, we first examined whether short interfering RNA (siRNA)-mediated knockdown of endogenous *DBC1* has any effect on p53 function. To avoid possible off-target effects caused by the *DBC1* RNAi, we used two different RNAi sequences that target different regions of the *DBC1* messenger RNA (mRNA). Thus, human osteosarcoma U2OS cells were transfected with the *DBC1*-specific siRNA#1 (*DBC1*-RNAi#1), *DBC1*-specific siRNA#2 (*DBC1*-RNAi#2) or a control siRNA (Control-RNAi). As shown in Fig. 4a, RNAi-mediated knockdown of *DBC1* expression had no obvious effect on p53 stability (lanes 2 and 3) but significantly reduced the expression levels of PUMA and BAX, two major transcriptional targets of p53. As expected, knockdown of *p53* expression by *p53*-specific siRNA (*p53*-RNAi) completely abolished the expression of both PUMA and BAX (lane 4), validating that expression of these two targets is indeed p53-dependent. These experiments demonstrate that inactivation of endogenous DBC1 leads to down-regulation of p53 activity.

Moreover, to demonstrate that DBC1 acts on p53 by repressing SIRT1 deacetylase activity, we tested whether inactivation of DBC1 indeed reduces acetylation levels of endogenous p53 by SIRT1 and, more importantly, whether these effects are reversed by inactivation of SIRT1 expression. Thus, these cells were transfected with the *DBC1*-specific siRNA#1 (*DBC1*-RNAi#1), *SIRT1*-specific siRNA (*SIRT1*-RNAi) or a control siRNA (Control-RNAi). As shown in Fig. 4b, RNAi-mediated knockdown of *DBC1* expression significantly reduced the acetylation levels of endogenous p53 (Ac-p53, bottom panel, lane 3). Notably, the reduction of p53 acetylation was completely reversed by concomitant knockdown of SIRT1 (Ac-p53, bottom panel, lane 4). Similar results were also observed with DBC1-mediated effects on PUMA and BAX by concomitant knockdown of *SIRT1* (PUMA and BAX, middle panel, lanes 3 and 4). Thus our data demonstrate that DBC1-mediated effects on p53 activation act mainly through SIRT1 *in vivo*.

To investigate the role of DBC1 in the stress response, we tested whether inactivation of DBC1 leads to inhibition of p53-dependent apoptosis on DNA damage. For this, U2OS cells were first transfected with either control or *DBC1*-specific siRNAs and then exposed to etoposide. Thirty hours later, the cells were stained with DAPI and apoptosis was examined by TUNEL staining. The DBC1-depleted cells were highly resistant to apoptosis, displaying only 8.8% apoptotic cells compared with 20.5% of cells transfected with the control siRNA (Fig. 4c and Supplementary Fig. 11). To confirm further the role of DBC1 in regulating p53-mediated apoptosis, we performed an apoptosis assay by using Annexin V staining followed by flow-cytometry analysis. Again, p53-mediated apoptosis was repressed in *DBC1* knockdown cells (Fig. 4d and Supplementary Fig. 12). As expected, inactivation of p53 in these cells completely abolished the apoptotic response by DNA damage. Notably, concomitant knockdown of *SIRT1* reversed the inhibitory effects on p53-dependent apoptosis by DBC inactivation. These data demonstrate that DBC1 is critically involved in regulating the p53-mediated apoptotic response by repressing SIRT1 function.

Our findings may have significant implications in the treatment of both metabolic-related disorders and cancer. Small molecule inhibitors of the SIRT1 deacetylase have been proposed as novel anticancer agents[22–24], presumably through activating the apoptotic response in cancer cells. On the other hand, activation of SIRT1 in mice also protects them against diet-induced obesity and insulin resistance, mainly through regulating metabolic pathways[25,26]. Although a role for mammalian SIRT1 in the regulation of lifespan has not been directly determined, the ability of resveratrol, a chemical found in red wine and other foods, to enhance SIRT1 activity and increase lifespan in lower organisms supports the feasibility of this approach in mammals[3,27–30]. Therefore, both inhibitors and activators of SIRT1 could be therapeutically beneficial by affecting different SIRT1-mediated regulatory pathways. It will be intriguing to know whether DBC1 has differential effects in regulating apoptotic responses versus metabolic pathways and whether manipulations of the DBC1–SIRT1 interaction will help to find more potent activators and/or inhibitors for SIRT1 activity.

METHODS SUMMARY

H1299, U2OS, 293 and HeLa cells were maintained in DMEM medium supplemented with 10% fetal bovine serum. H1299 and 293 cells were transfected with plasmid DNA by the calcium phosphate protocol or the siRNA RNA duplex, as indicated in the relevant figures, by Lipofectamine2000 according to the manufacturer's protocol. *In vitro* deacetylation assays were performed as previously described. Purified acetylated p53 was incubated with purified SIRT1 and DBC1 as indicated at 30 °C for 1 h in the presence of 50 μM NAD, and reactions were resolved by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and analysed by western blot. To immunoprecipitate the ectopically expressed Flag-tagged proteins, transfected cells were lysed 24 h post-transfection in Flag-lysis buffer and precipitated using anti-Flag monoclonal antibody-conjugated M2 agarose beads. The eluted material was resolved by SDS–PAGE and detected by antibodies as indicated. The endogenous SIRT1 complex was purified from HeLa cells by using the SIRT1-CT-specific antibody for immunoprecipitation. Interacting proteins were identified by mass spectrometry. To analyse the SIRT1 complex, 50 μl of M2-eluted Flag-SIRT1 containing approximately 12.5 μg of total purified Flag-SIRT1 was fractionated by size-exclusion chromatography. GST pull-downs were performed as previously described, using recombinant fragments of GST–SIRT1 or GST–control to pull-down ^{35}S-methionine-labelled DBC1. Direct *in vitro* interaction was detected by autoradiation after the reactions were resolved by SDS–PAGE. Dual luciferase reporter assays to determine transcriptional activity were performed in H1299 cells according to the manufacturer's guidelines 24 h post-transfection. The ablation of DBC1 was performed by transfection of the U2OS cells with either of two siRNA duplex oligonucleotides which covered mRNA regions of nucleotides 582–602 (amino acids 55–61) and nucleotides 2097–2115 (amino acids 560–565) of *DBC1*, respectively, by using Lipofectamine2000 according to the manufacturer's protocol. *SIRT1* RNAi, *p53* RNAi and Control RNAi were used and transfected according to the manufacturer's guidelines. Annexin V-FITC and TUNNEL staining were performed according to the manufacturer's guidelines.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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LETTERS

Structure and mechanism of the M2 proton channel of influenza A virus

Jason R. Schnell[1] & James J. Chou[1]

The integral membrane protein M2 of influenza virus forms pH-gated proton channels in the viral lipid envelope[1]. The low pH of an endosome activates the M2 channel before haemagglutinin-mediated fusion. Conductance of protons acidifies the viral interior and thereby facilitates dissociation of the matrix protein from the viral nucleoproteins—a required process for unpacking of the viral genome[2]. In addition to its role in release of viral nucleoproteins, M2 in the trans-Golgi network (TGN) membrane prevents premature conformational rearrangement of newly synthesized haemagglutinin during transport to the cell surface by equilibrating the pH of the TGN with that of the host cell cytoplasm[3]. Inhibiting the proton conductance of M2 using the anti-viral drug amantadine or rimantadine inhibits viral replication[4–7]. Here we present the structure of the tetrameric M2 channel in complex with rimantadine, determined by NMR. In the closed state, four tightly packed transmembrane helices define a narrow channel, in which a 'tryptophan gate' is locked by intermolecular interactions with aspartic acid. A carboxy-terminal, amphipathic helix oriented nearly perpendicular to the transmembrane helix forms an inward-facing base. Lowering the pH destabilizes the transmembrane helical packing and unlocks the gate, admitting water to conduct protons, whereas the C-terminal base remains intact, preventing dissociation of the tetramer. Rimantadine binds at four equivalent sites near the gate on the lipid-facing side of the channel and stabilizes the closed conformation of the pore. Drug-resistance mutations are predicted to counter the effect of drug binding by either increasing the hydrophilicity of the pore or weakening helix–helix packing, thus facilitating channel opening.

M2 is a 97-residue single-pass membrane protein that has its amino and carboxy termini directed towards the outside and inside of the virion, respectively; it is a homotetramer in its native state[8,9]. The four transmembrane helices form a channel in which His 37 is the pH sensor and Trp 41 is the gate[6,10,11]. The adamantane-based drugs amantadine and rimantadine, which target the M2 channel, have been used as first-choice antiviral drugs against community outbreaks of influenza A viruses for many years, but resistance to the adamantanes has recently become widespread. Many structural models of this channel have been built, based on sequence analysis, mutagenesis and solid-state NMR[8,11,12]. Many of these studies have been done on inherently unstable transmembrane-only constructs, however, leading to conflicting structural conclusions.

Although the transmembrane-only peptide fails to form a stable tetramer, a construct of residues 18–60 (M2(18–60)), which includes 15 residues of the C terminus in addition to the transmembrane region, forms a stable tetramer in dihexanoyl-phosphatidyl-choline (DHPC) detergent micelles and yields high-resolution NMR spectra (Supplementary Fig. 1). In the closed conformation at pH 7.5, M2(18–60) is a homotetramer in which each subunit has an unstructured N terminus (residues 18–23), a channel-forming transmembrane helix (residues 25–46), a short flexible loop (residues 47–50) and a C-terminal amphipathic helix (residues 51– 59). The transmembrane helices assemble into a four-helix bundle with a left-handed twist angle of ~23° and a well defined pore (Fig. 1). A ring of methyl groups from Val 27 constricts the N-terminal end of the pore to ~3.1 Å (inner diameter). In agreement with proposed models[11,13], His 37 and Trp 41 are inside the pore. A three-bond, ^{15}N–$^{13}C\gamma$ scalar coupling ($^{3}J_{NC\gamma}$) value of 1.5 Hz (Supplementary Table 1) shows the His 37 χ_1 rotamer to be predominantly *trans*, but with significant rotameric averaging. The χ_1 of Trp 41 is essentially locked in the *trans* position, as determined by a $^{3}J_{NC\gamma}$ of 2.6 Hz, whereas the χ_2 is also fixed at around −120° by the side chain Hε1–Nε1 dipolar coupling and nuclear Overhauser effects (NOEs). The Trp 41 indole rings are at van der Waals distance from each other, prohibiting passage of water or ions (Fig. 1c). The indole Hε1 of one subunit is on average 3.5 Å from the Asp 44 carboxyl carbon of the adjacent subunit. The two residues can form an intermolecular hydrogen bond that stabilizes the closed Trp 41 gate. The side chain of Arg 45 probably participates in an intermolecular interaction with Asp 44. These findings are consistent with the increased pH-modulated activity of channels in which asparagine has replaced Asp 44 (ref. 14).

The C-terminal end of the channel extends into a loop (residues 47–50) that connects the transmembrane domain to the C-terminal amphipathic helix. Residual dipolar couplings (RDCs) and intra- and inter-monomer NOEs show that the amphipathic helices lie roughly perpendicular (~82°) to the transmembrane helices and assemble head-to-tail using a right-handed packing mode to form the base of the channel. The orientation and amphipathic character of the amphipathic helices suggest that the C-terminal base lies on the surface of the membrane.

Residues 47–50 give no NOE peaks and do not have a stable, hydrogen-bonded structure in the detergent micelles used in our work. We believe that this segment adopts a more stable conformation in the viral membrane because Cys 50, which we mutated to serine to avoid disulphide formation, is normally palmitoylated[15]. Modelling shows that extending the transmembrane helix to Phe 48 would place residue 50 facing the membrane, allowing for insertion of the palmitoyl acyl chain into the lipid bilayer. This minor rearrangement would also move the amphipathic helices closer to the transmembrane domain.

Drug binding stabilizes the closed conformation. On addition of drug, the resonances of residues 43–46 at the C terminus of the channel, which are severely exchange-broadened in the drug-free sample, became significantly sharper and more homogeneous (Supplementary Fig. 2). The protein–drug NOEs collected from four different NOE spectroscopy (NOESY) spectra (Supplementary Fig. 3) place the binding site between adjacent helices at the C-terminal end of the transmembrane domain near the Trp 41 gate, on the membrane side of the channel (Fig. 1d). We could not detect

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drug NOEs in other parts of the protein, including the widely proposed drug-binding site in the pore of the channel. The amine headgroup of rimantadine is in contact with the polar side chains of Asp 44 and Arg 45, and with the indole amine of Trp 41. The side chains of Ile 42 from one helix and Leu 40 and Leu 43 from another helix form the hydrophobic walls of the binding pocket that interact with the adamantane group of rimantadine. Thus, rimantadine covers a unique polar patch in the otherwise hydrophobic environment of the transmembrane domain. Interactions between rimantadine and the channel are consistent with structure–activity relationships of the adamantane group[16]. In particular, the basic nitrogen group and size limits at the methyl site are critical. These requirements are the result of the interactions with Asp 44 and the small hydrophobic pocket around Ile 42, respectively.

Water NOEs measured in the 110-ms ^{15}N-separated NOESY experiments give a clear picture of water distribution relative to the channel (Fig. 2). The lipid-facing surface of the transmembrane region is largely protected from water by the DHPC micelle. In the closed channel pore, the Val 27 ring at the N terminus and the Trp 41 gate at the C terminus essentially block water from freely diffusing into the pore from either side of the membrane. Within the transmembrane region, only the amides of Ser 31 and Ile 32 have NOE crosspeaks at the chemical shift of water, probably corresponding to the hydroxyl proton of Ser 31 in exchange with water. A polar residue is present at position 31 in all sequenced variants of M2, suggesting that proton conduction requires water to be bound to this site. This water may serve to bridge the proton relay from the N-terminal end of the pore to the His 37 pH sensor. Water was detected at the C terminus of the transmembrane region, beginning at Arg 45. The Hε1 of the Trp 41 indole ring, which points towards the C-terminal side of the pore, also has a strong NOE to water, indicating that the base of the channel is accessible to bulk water.

Lowering the pH from 7.5 to 6.5 broadens most of the NMR resonances corresponding to the transmembrane helix (Fig. 3a). The resonance broadening could not be attributed to protein aggregation, because the self-diffusion coefficients were essentially unchanged between pH 7.5 and pH 6.5. Thus, activation of the channel is coupled to increased conformational exchange in the transmembrane domain. In contrast, the resonances of the amphipathic helices are essentially unaffected by lowering the pH, indicating that the C-terminal base of the tetramer remains intact as the channel opens.

In addition to destabilizing helix–helix packing in the transmembrane domain, channel activation must also correlate with increased dynamics of the Trp 41 gate. Because the indole amide resonance of Trp 41 remained strong as the pH was lowered from 7.5 to 6.0, it serves as a useful NMR probe for monitoring opening of the channel. We compared the millisecond timescale dynamics of the Trp 41 indole ring between the closed and open states by carrying out relaxation-compensated Carr–Purcell–Meiboom–Gill (CPMG) experiments[17] at pH 7.5, pH 7.0 and pH 6.0. A two-site exchange model fits the dependence of ^{15}N relaxation caused by chemical shift exchange on the frequency of refocusing ($1/\tau_{cp}$) of chemical shift evolution (Fig. 3b), implying that the gate switches between two configurations at any given pH. As the pH was lowered from 7.5 to 6.0, the rate of fluctuation increased by more than fourfold (Fig. 3b), indicating that channel activation 'unlocks' the gate. Adding rimantadine to the channel at an intermediate pH of 7.0 slowed the timescale of the gate motion to nearly that of a drug-free gate at pH 7.5 (Fig. 3c). These results confirm that the reconstituted channels in the

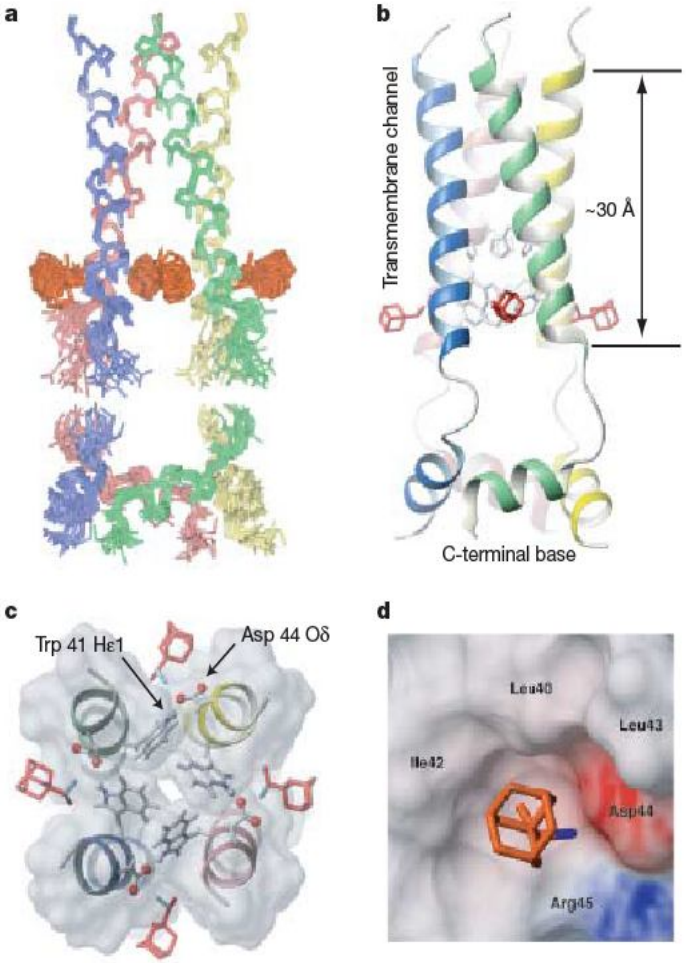

Figure 1 | Structure of the M2 channel. **a**, An ensemble of 15 low-energy structures derived from NMR restraints. Because residues 47–50 are unstructured, the transmembrane helices (residues 25–46) and the amphipathic helices (residues 51–59) are superimposed separately. The backbone r.m.s. deviations for the transmembrane and amphipathic helices are 0.30 Å and 0.56 Å, respectively. **b**, A ribbon representation of a typical structure from the ensemble in **a**, showing the left-handed packing of the transmembrane helices, right-handed packing of the amphipathic helices, the side chains of His 37 and Trp 41, and the drug rimantadine (coloured in red). **c**, A close-up view from the C-terminal side of the channel showing the Trp 41 gate and how it is stabilized by the inter-monomer hydrogen bond between Trp 41 Hε1 of one transmembrane helix and Asp 44 carboxyl of the adjacent transmembrane helix. **d**, The surface representation of the rimantadine-binding pocket, showing the Asp 44, the indole amine of Trp 41, and Arg 45, which form the polar patch, as well as the hydrophobic wall composed of Leu 40, Ile 42 and Leu 43.

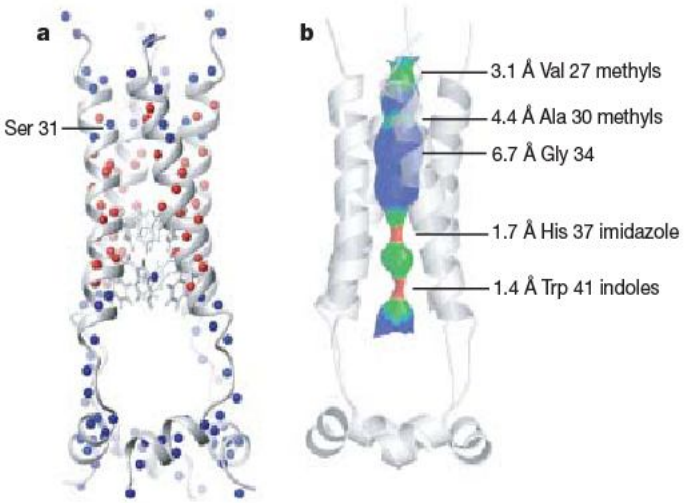

Figure 2 | Water accessibility of the M2 channel. **a**, Distribution of water NOEs relative to the structure. Amide protons coloured in blue have a NOE crosspeak to water. Those that do not are coloured red. **b**, The pore surface calculated using the program HOLE. The region of the channel coloured in green is only wide enough to allow passage of a water molecule, whereas the blue portion can accommodate two or more water molecules. The orange region is too narrow to allow any ions to pass through.

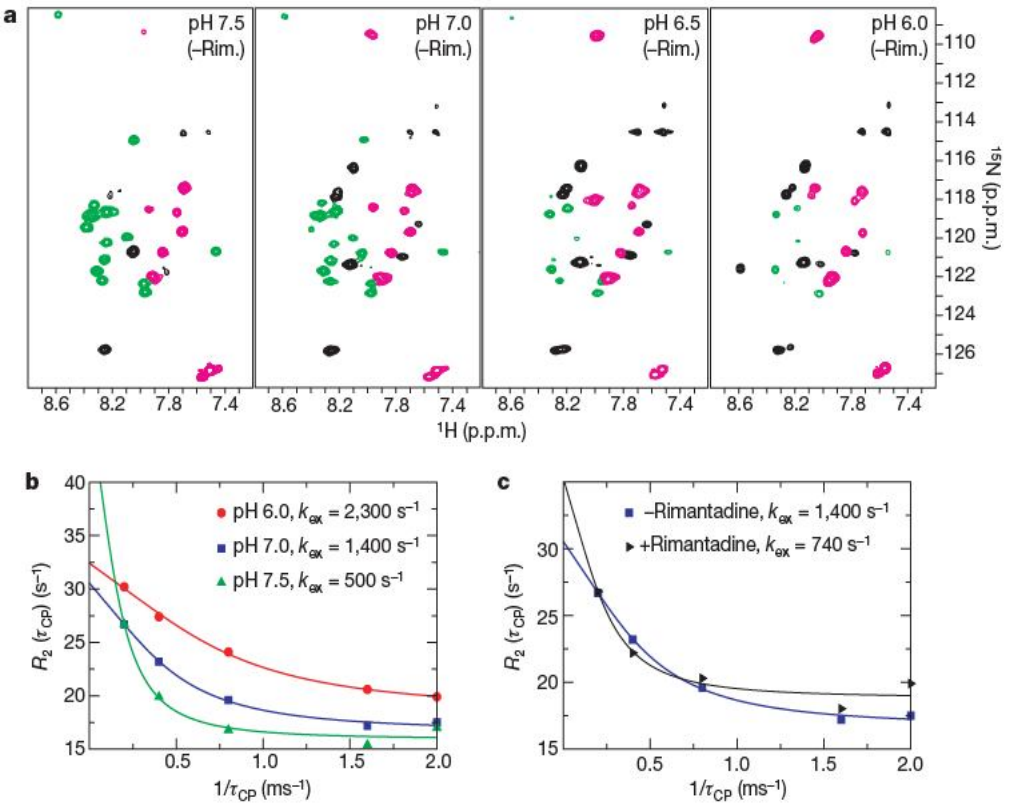

Figure 3 | Low-pH-induced destabilization of the channel and opening of the Trp 41 gate. **a**, ^{1}H-^{15}N TROSY spectra of reconstituted M2(18–60) tetramer at pH 6.0, 6.5, 7.0 and 7.5, in the absence of rimantadine (−Rim.), recorded at 500 MHz ^{1}H frequency and 30 °C. Green, transmembrane helix; pink, amphipathic helix; black, N-terminal loop. **b**, The ^{15}N R_2 (pure $R_2 + R_{ex}$) of the Trp 41 Nε1 as a function of the frequency of refocusing (1/τ_{cp}) of chemical shift evolution obtained at pH 7.5, 7.0 and 6.0, showing faster timescale motion of the Trp 41 gate as the channel is activated. **c**, Comparison between $R_2(\tau_{cp})$ at pH 7.0 in the absence (blue) and presence (black) of rimantadine, demonstrating that the drug slows down the gate flipping at this pH.

NMR sample are pH-gated, and are consistent with the location of the rimantadine site proximal to the gate.

The structure of the M2 proton channel thus reveals a simple yet effective gating. The tight packing of the four transmembrane helices brings the bulky indole rings of Trp 41 into van der Waals contact to form the channel gate. The gate is further stabilized by inter-subunit hydrogen bonds with Asp 44. Lowering the pH protonates the imidazole rings of His 37, destabilizing helix–helix packing by electrostatic repulsion. This conformational rearrangement breaks interactions between Trp 41 and Asp 44 and allows the gate to flip open. A pair of conserved N-terminal cysteines have been shown to form intermolecular disulphides *in vivo*[9]. Thus, the transmembrane helices are tethered at one end by N-terminal disulphides and at the other end by the C-terminal base, ensuring that destabilization of the four-helix bundle during channel activation does not cause dissociation of the tetramer (Fig. 4). Indeed, truncation of the amphipathic helix results in channels that rapidly lose channel activity[18].

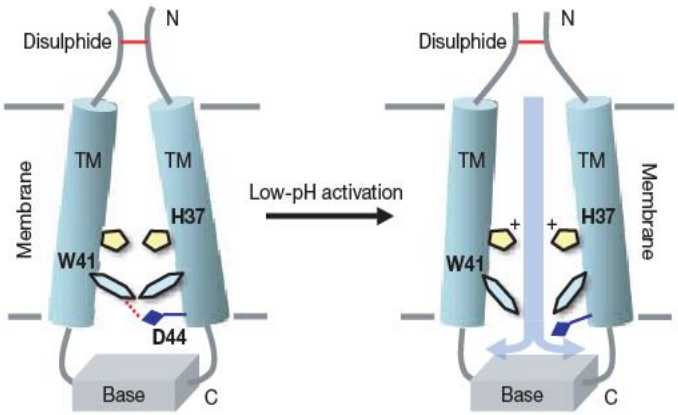

Figure 4 | Schematic illustration of M2 channel activation. At high pH, the transmembrane (TM) helices are packed tightly and the tryptophan gate is locked through intermolecular interactions with Asp 44. At low pH, protonation of the His 37 imidazoles destabilizes the transmembrane helix packing, allowing hydration of the channel pore and proton conductance. The C-terminal base of the tetramer and N-terminal disulphide bonds keep the channel from completely disassembling. For clarity, only two of the four monomers are shown.

The discovery of the external drug-binding site was unexpected. Drug-resistance mutations seemed to suggest that the drug-binding site was inside the pore, because, in early models of the channel, residues that lead to drug resistance were predicted to be pore-lining. The known mutations that confer drug resistance are L26F, V27A, A30T, S31N, G34E and L38F. Mapping these residues onto the structure (Supplementary Fig. 7) reveals that Val 27, Ala 30 and Gly 34 are pore-lining, but that Leu 26, Ser 31 and Leu 38 are in the helix–helix packing interface. Moreover, these mutations are spread out over more than three turns of the transmembrane helix, covering a distance much larger than the dimensions of amantadine or rimantadine. The authors of ref. 19 pointed out that having a cork-plugging-the-bottle model is insufficient to explain all the results of electrophysiology studies. For example, drug inhibition is more effective when applied to the closed channel than to the open channel, which is not expected of a pore-blocking mechanism[5]. Several of the drug-resistance mutations in pore-lining residues have been shown to retain drug binding[20]. Although a pore blocker is expected to fit tightly in the pore, channel inhibition is unusually tolerant of modifications to the adamantane scaffold[16]. Together, the above observations suggest an allosteric inhibition mechanism.

Is the external binding site consistent with all drug-resistance mutations? Although the exact structural effects of resistance mutations are difficult to predict, what they do have in common is that they either perturb the helix–helix interface (L26F, V27A, S31N, L38F) or increase the hydrophilicity of the pore (A30T, G34E).

From this observation, and from detection of a conformational exchange among multiple states at lowered pH, we propose an allosteric inhibition mechanism that can account for all of the mutations. In our model, drug binding makes the closed channel harder to open, whereas drug-resistance mutations destabilize the closed channel, making it easier to open. Replacing Val 27 with alanine enlarges the N-terminal opening and weakens helix–helix packing, and therefore may facilitate channel opening. Ala 30 and Gly 34 are inside the pore, and replacing them with threonine and glutamate, respectively, may facilitate pore hydration, and, in turn, channel opening. Leu 26, Ser 31 and Leu 38 are helix–helix interface residues; their mutations probably perturb helix–helix packing and lower the energetic cost of channel opening.

Why, then, are no drug-resistant mutations observed near the drug-binding site? In fact, few mutations are ever observed in this region of the channel (drug-resistant or otherwise), which is not surprising owing to the functional constraints placed on these residues in proximity to the channel pH sensor (His 37) and the channel gate (Trp 41). As the structure illustrates, intermolecular contacts between Asp 44 and Arg 45 form an integral part of the channel gate, along with Trp 41. The residues that form the hydrophobic walls of the binding pocket—Leu 40, Ile 42 and Leu 43—are on the lipid face of the channel and must retain hydrophobicity for membrane partitioning. To accommodate the Trp 41 indole rings within the channel, the helices splay slightly at the C terminus of the transmembrane domain, and interhelical contacts below Leu 38—with the exception of the Trp 41 side chains—are no longer important for channel assembly. Thus, residues essential to channel assembly are in the N-terminal half of the transmembrane helix, exactly where drug-resistance mutations occur.

Binding from the membrane side is consistent with the high membrane partition coefficient of adamantane drugs, which effectively concentrates them in the membrane and lowers their level in the aqueous phase[21,22]. Adamantanes interact with a number of other ion channels, including viroporins from hepatitis C[23], the potassium channel Kcv of the chlorella virus PBCV-1 (ref. 24) and the human NMDA receptors[25]. Hanatoxin, an allosteric inhibitor of voltage-gated K^+ channels with a high membrane partition coefficient, also has an external binding site[26,27]. Membrane-side binding may thus be a feature of many channel inhibitors. This mode of inhibition could be advantageous for drug design because drug molecules are typically much larger than hydrated ions selected by ion channels, and therefore the energy barrier for the drug to find a blocking site inside the channel pores would be much higher than targeting a functional site from the membrane side of the channel.

Note added in proof: We note that, in a separate X-ray study of the transmembrane domain of M2, an electron density, which was proposed to be from amantadine, was observed inside the channel pore (see ref. 28).

METHODS SUMMARY

The M2(18–60) polypeptide construct was expressed as a C-terminal fusion to bacterial trpLE with an N-terminal His_9 tag in the pMM-LR6 vector[29]. The M2(18–60) tetramer was reconstituted by dissolving peptide in a solution containing 50 mM sodium phosphate, 6 M guanidine HCl and 150 mM DHPC, dialyzing against a solution containing 40 mM sodium phosphate (pH 7.5) and 30 mM glutamate, and concentrating. Rimantadine was added to the reconstituted protein. The final NMR sample used for structure determination contained 0.75 mM M2(18–60) (monomer), ~300 mM DHPC and 40 mM rimantadine. Given that the DHPC has an aggregation number of 27 (ref. 30) and the strong partition coefficient of rimantadine in phospholipids (rimantadine aqueous solubility is very low, ~50 μM), locally, there are about four rimantadine molecules per micelle compartment in which the channel resides.

The NMR protocol used was similar to that described previously[31]. An extensive set of structural restraints (including 230 × 4 intra- and 27 × 4 inter-molecular distance restraints derived from NOEs, 27 × 4 orientation restraints from residual dipolar couplings (RDCs), and 23 × 4 side-chain rotamers from three-bond scalar couplings) were used to generate an ensemble of 15 low-energy structures with a backbone root mean square (r.m.s.) deviation of 0.30 Å for the channel region and of 0.89 Å for all structured regions (Fig. 1a). The refinement statistics and NMR-derived restraints are summarized in Supplementary Table 1. Structure calculation was accomplished in two steps, in which the overall tetramer conformation was first defined by NOE-derived distance restraints and *J*-coupling-derived dihedral restraints using a high-temperature simulated annealing protocol, and was subsequently refined against RDCs at low temperature.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Contributions J.R.S. and J.J.C. designed research, performed research, analysed data and wrote the paper.

Author Information The structures have been deposited in the Protein Data Bank under the accession number 2RLF. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details accompany full-text HTML version of the paper on www.nature.com/nature. Correspondence and requests for materials should be addressed to J.J.C. (james_chou@hms.harvard.edu).

LETTERS

Structural basis for the function and inhibition of an influenza virus proton channel

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The M2 protein from influenza A virus is a pH-activated proton channel that mediates acidification of the interior of viral particles entrapped in endosomes. M2 is the target of the anti-influenza drugs amantadine and rimantadine; recently, resistance to these drugs in humans, birds and pigs has reached more than 90% (ref. 1). Here we describe the crystal structure of the transmembrane-spanning region of the homotetrameric protein in the presence and absence of the channel-blocking drug amantadine. pH-dependent structural changes occur near a set of conserved His and Trp residues that are involved in proton gating[2]. The drug-binding site is lined by residues that are mutated in amantadine-resistant viruses[3,4]. Binding of amantadine physically occludes the pore, and might also perturb the pK_a of the critical His residue. The structure provides a starting point for solving the problem of resistance to M2-channel blockers.

Although models of the M2 channel have been proposed on the basis of mutagenesis[5], molecular dynamics[6–8] and spectroscopic studies[9–11], high-resolution crystallographic or solution NMR structures have not been available. We therefore solved the structure of a peptide spanning the transmembrane helix of M2 (M2TM)[11–14]. Like the full-length protein, M2TM associates into a tetrameric four-helix bundle that binds amantadine[9,13] and conducts protons[10]. Several functional variants of M2TM[15] were screened for their ability to form diffraction-quality crystals. A crystal form that diffracts to 2.0 Å resolution was obtained from a peptide, in which Ile 33 was changed to selenomethionine (I33-SeMet), at pH 7.3 in the absence of amantadine. The peptide crystallizes with six octyl-β-D-glucopyranoside detergent molecules that form a bilayer-like environment into which M2TM tetramers are embedded (Supplementary Fig. 1). A second mutant, G34A, was crystallized at a lower pH (pH 5.3) in the presence of amantadine (diffraction limit, 3.5 Å resolution). The two structures are very similar (root mean squared deviation, r.m.s.d., for all atoms is 1.8 Å), with the primary differences lying near the carboxy-terminal region of the helices.

In both structures the tetrameric M2TM helices form a left-handed, parallel bundle (Fig. 1a) that resembles a conical frustum (a truncated cone), with the narrow amino-terminal end facing the exterior of the viral envelope. Each helix is preceded by a polar, highly conserved sequence, Ser–Ser–Asp (Fig. 1a), four copies of which form a narrow, solvent-filled pore lined by Ser hydroxyls and main-chain carbonyl groups. Protons pass through this extra-viral vestibule *en route* to the section of the transmembrane pore formed by the four-helix bundle (residues 25–45, Fig. 1a). The N-terminal half of the channel has nearly exact four-fold rotational symmetry; the helices are tilted by 35° ± 2° with respect to the central axis of the bundle, which is within the range of 30° to 40° observed by solid-state NMR (ssNMR) for both M2TM[14,16] and a longer fragment of the protein[14]. The pore is most constricted near Val 27; beyond this point it opens to create an aqueous cavity lined by small residues (Ala 30, Ser 31 and Gly 34), reaching a maximal diameter of 9 Å at Gly 34 near the centre of the bilayer. Similar large aqueous cavities are often observed near the centre of channel proteins, where they seem to minimize the thermodynamic cost of bringing a charged ion to the centre of a bilayer[17]. The channel constricts again at the critical pH-gating residues His 37 and Trp 41.

Full-length M2 is inhibited by amantadine with a cooperativity factor of 1.0 (ref. 18); the protein binds a single drug molecule per tetramer[19]. The electron-density map from crystals grown in the presence of amantadine shows strong density of the same size and shape as that of this drug molecule (Fig. 1b). The drug is surrounded by residues (including Val 27, Ala 30, Ser 31 and Gly 34) that are mutated in clinical isolates of amantadine-resistant viruses[1,4,20]. The same hotspot for amantadine resistance was pinpointed in positional scanning mutagenesis of the full-length protein[2,5] (Fig. 1b, c). Furthermore, residues that can be mutated without affecting amantadine inhibition[2,5] lie more distal to the drug-binding site at membrane-accessible and C-terminal locations (including Leu 38 to Asp 44)[2,5]. The structure is in agreement with electrophysiological studies of full-length M2 that showed that the rate of channel block is approximately 10^5-fold slower than that expected for diffusion of a small molecule into a channel with a large extracellular opening[18]. The highly restricted vestibule helps to explain the slow kinetics of entry of the drug, which might enter the site by means of rare conformational changes or laterally from the bilayer phase.

The drug-binding site is nearly identical (r.m.s.d. = 0.4 Å over all atoms of Val 27, Ala 30, Ser 31 and Gly 34) between the amantadine-bound (low pH) and the drug-free (high pH) crystal structures; this is consistent with amantadine's known ability to inhibit between pH 5.0 and pH 8.0 (refs 9, 18, 19). Given the resolution of the structure, two orientations of the drug are possible: the amine group could either point towards the viral exterior or point inward, where it would be hydrated in the aqueous pore (Fig. 1b). The drug fits the density better in the inward orientation with its large, apolar group snugly fit into the N-terminal end of the aqueous cavity (Fig. 1b, c). The polar end of the drug projects towards, but does not directly contact, His 37. This binding mode is consistent with the fact that if the amino group of amantadine is substituted with structurally diverse bulky secondary alkylamines, inhibitory activity is retained[21]. Long-range interactions between the ammonium group and His 37

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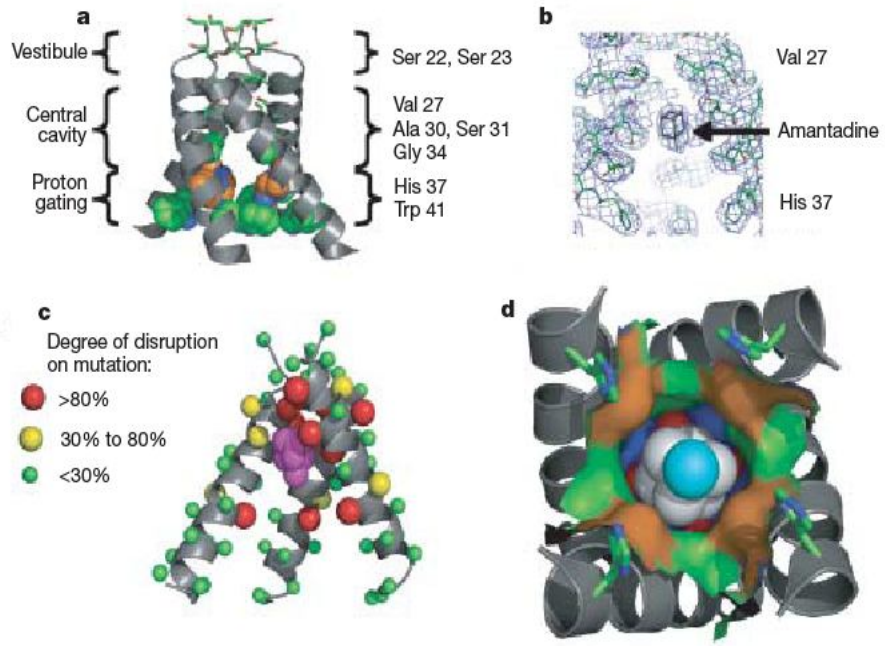

Figure 1 | Crystal structure of the M2 proton channel from the influenza A virus. **a**, The most critical residues identified by site-directed mutagenesis[5] line the pore. Gly 34, His 37 and Trp 41 are shown in space-filling spheres (carbon atoms of His 37 are coloured tan), whereas the side chains of the other critical residues are shown as sticks. **b**, Omit map ($2F_o - F_c$, contoured at 1σ) showing electron density in the amantadine-binding region. **c**, Positions of previously described Cys mutations[5] that disrupt the ability of amantadine to block the channel are shown by red balls (>80% disruption), yellow balls (30% to 80%) and green balls (no significant disruption) in full-length M2. Amantadine is shown in magenta. The front helix was removed for clarity. **d**, Structure of amantadine (nitrogen in cyan and carbon in white) inside the binding site showing the surface associated with residues Val 27 (red surface), Ala 30 (green), Ser 31 (blue) and Gly 34 (orange).

might also account for shifts in this residue's pK_a that accompany the binding of the drug[9].

The recent, marked rise in amantadine resistance has been associated with a single mutation, S31N (ref. 20). In the 2005–2006 flu season, resistance reached more than 90%, and 99.9% of the resistant viruses collected worldwide (1,059 of 1,060) had the S31N mutation[1]. Asn can be modelled in a low-energy rotamer at position 31 of the crystal structure, resulting in extensive hydrogen bonding between the Asn carboxamides (Supplementary Fig. 2). The side chains form a carbonyl-lined hole that can accommodate one or more water molecules, explaining the retention of proton-channel activity in this mutant. This mutation also constricts the size and increases the polarity of the amantadine-binding site—features that should interfere with the binding of the large hydrophobic adamantane group. Furthermore, Ser-to-Asn mutations stabilize transmembrane helix–helix association[22]. Thus, the S31N mutation seems to be particularly fit in terms of its ability to allow proper insertion, assembly and function of M2 in membranes, while escaping inhibition by amantadine. Interestingly, although S31N constricts the amantadine-binding site in the N-terminal half of the transmembrane pore, this mutation does not fill the aqueous pore adjacent to His 37 and Trp 41—functionally essential residues conserved in all influenza A and B viruses. New drugs that bind this region might inhibit both Ser 31 and Asn 31 variants of M2, and they should also be less susceptible to the development of new resistance.

The crystal structures at neutral and low pH provide insight into the role of His 37 and Trp 41 in the conduction of protons. These residues lie near the channel exit within the wide end of the bundle. In the low-pH structure, all four helices are straight and diverge from a point where the helical axes most closely approach the central four-fold symmetry axis, which occurs in the vicinity of Val 27. This divergence creates to a large opening near His 37 and Trp 41. However, the pore of the neutral-pH form is smaller and less symmetric; helix D has a gradual bend of 15° near Gly 34, allowing Trp 41_D (the subscript refers to helical subunit D) to interact with His 37_C in an edge-on aromatic interaction (Fig. 2a, b) previously predicted from spectroscopic[11] and ssNMR[23] measurements. This conformation is further stabilized by an interhelical salt bridge between Arg 45_D and Asp 44_C (Fig. 2a, b). In contrast, the A and B helices are more similar to the symmetrical low-pH form. They are nearly completely straight and consequently diverge too far to allow interhelical His–Trp and Arg–Asp interactions (Fig. 2a, c).

The finding of multiple conformations at the gating end of the channel is consistent with the known pH-dependent dynamic and functional properties of M2. The neutral form was crystallized at pH 7.3, at which the four His 37 residues should be in a mixed protonation state on the basis of their known pK_a values in M2TM (8.2, 8.2, 6.3 and <5)[10]. The pH-dependent structural variability seen between the low-pH and neutral structures is consistent with disulphide crosslinking studies in the full-length protein that demonstrated a selective increase in C-terminal contacts at a neutral pH (ref. 24). Also, spectroscopic measurements of M2TM in

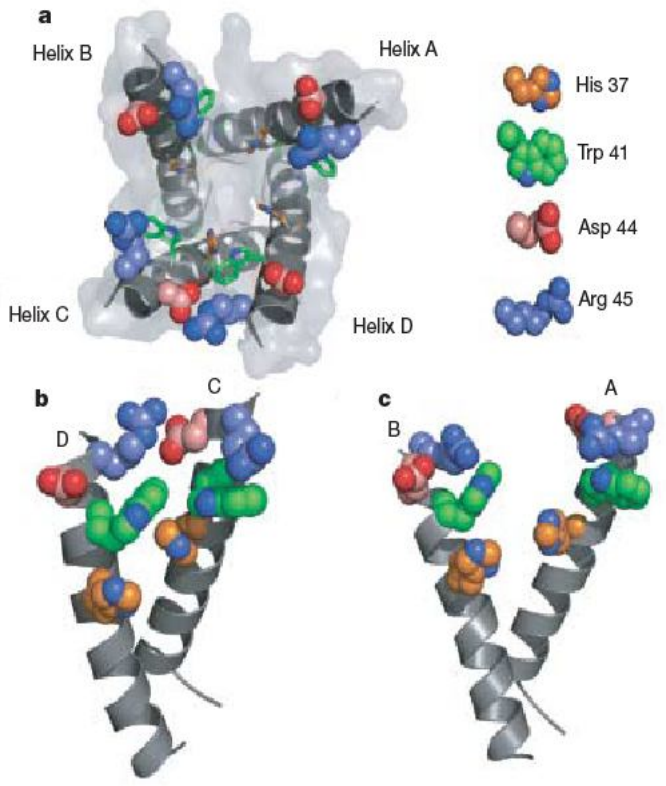

Figure 2 | Asymmetric structure of the C-terminal His/Trp gate of M2TM. **a**, Crystal structure of the tetramer viewed from the inside of the virus, with the helices labelled A–D. **b**, In the C–D interface, Asp 44 and Arg 45 form a salt bridge, and His 37 and Trp 41 engage in an inter-subunit edge-on aromatic interaction. **c**, These residues do not interact at the A–B interface.

phospholipid bilayers have revealed that the helices can be straight or bent near Gly 34 (ref. 25). The curve in helix D occurs in the vicinity of Gly 34, which is one turn apart from a pair of small side chains, Ala 30 and Ser 31. Similar sequences are believed to mediate bending of transmembrane helices during the gating of potassium channels[26]. Interestingly, the low-pH form was solved using the G34A mutation; if this region is indeed part of a hinge, this mutation might help to stabilize an open conformation with straight rather than curved helices.

To explore the functional implications of the conformational differences in the subunits of the neutral-pH form, we superimposed chains A, B, C and D of M2TM onto B, C, D and A, respectively, and repeated this procedure for each of the remaining cyclic permutations (Fig. 3a). The resulting family of four tetramers superpose extremely well at the N terminus of the bundle, but become increasingly divergent at the C terminus. The four copies of helix A form a bundle, designated A4, in which the straight helices diverge near the C terminus as in the low-pH structure (Fig. 3b, r.m.s.d. = 1.5 Å over all atoms). At the other extreme, the D4 bundle, which is almost completely closed at the C terminus of the bundle, is similar to a coiled-coil (Fig. 3c–e). The A4 and D4 models suggest a minimal mechanism for the pH-dependent activation of the channel (Fig. 4). At high-pH values, the channel would be mostly in a D4-like 'closed' state, whereas a decrease in pH would trigger electrostatic repulsions between multiple protonated His residues to a more open A4-like state with improved hydration of the charged His side chains[6,7].

This mechanism is in agreement with the effects of mutations on the pH-dependent activation of the channel. The His_C–Trp_D and Arg_D–Asp_C interactions seen in the helix C–D interface of the crystal structure (Fig. 2b) and at each interface in the D4 bundle (Fig. 3e) seem to help to stabilize the closed conformation. Mutating Asp 44 to Asn caused a large increase in the activity of M2 (ref. 27); presumably, this mutation shifts the equilibrium to the open conformation by disrupting the Asp–Arg salt bridge. Furthermore, Trp 41, which helps trap protons within an acidified virus[2], is found to block access of His 37 from the side of the D4 tetramer facing the inside of the virus (Fig. 3e).

The structure-based model for the open state (A4 tetramer) is also consistent with the electrophysiological properties of M2. Although M2 is considered to be a channel, it has a very low maximal conductance rate of less than 10^4 protons per second (ref. 28)—much lower than that of typical ion channels, which usually have maximal rates of 10^5 to 10^7 ions per second. The slow rate of M2 is consistent with the restricted channel vestibule and argues against a highly populated open state with either a very large pore or an uninterrupted organized 'wire' of water molecules. Furthermore, His 37 lies about two-thirds of the way through the transmembrane electrical field[28,29], which also argues against a large (>10 Å) uninterrupted pore leading from the outside of the virus to His 37 that would place this residue near the beginning of the transmembrane potential gradient. Instead, the highly restricted N-terminal vestibule might contribute to proton selectivity. N-terminal motions would allow protons to penetrate into the aqueous pore by means of transient hydrogen-bonded chains of water molecules, whereas it might be very difficult for hydrated sodium or potassium to penetrate this region. Indeed, amantadine-sensitive variants in which Val 27, which lines the most constricted point in the pore, is mutated to Ser, Thr or Cys have increased conductance and/or compromised ion selectivity[5,30].

Earlier predictions are in reasonable accord with the crystallographic structures in terms of many of the overall features of the channel. The r.m.s.d. between experimentally restrained models on the basis of site-directed mutagenesis and conformational searching[5,15] is 3 Å to 4 Å, and these models accurately predicted the location of critical residues in the pore. The helix-crossing angles of the crystallographic structures are within the range of angles detected by ssNMR studies of M2TM[14,16] in buffered solution. Early attempts to define models on the basis of restraints from ssNMR were complicated by conformational averaging[9,10,25]. However, a recent study of the amantadine complex at high pH (ref. 25) showed a conformation for the helical monomer in excellent agreement with the bent helix D of the crystal structure (15° versus 11° by ssNMR[25]). A model based on these ssNMR angular restraints is consistent with the overall features of the crystallographic structures and the D4 model (all-atom r.m.s.d. = ~3.0 to ~3.3 Å).

The crystallographic structures are in excellent agreement with a wide body of functional and spectroscopic data and provide a basis for the design of new inhibitors that target amantadine-resistant

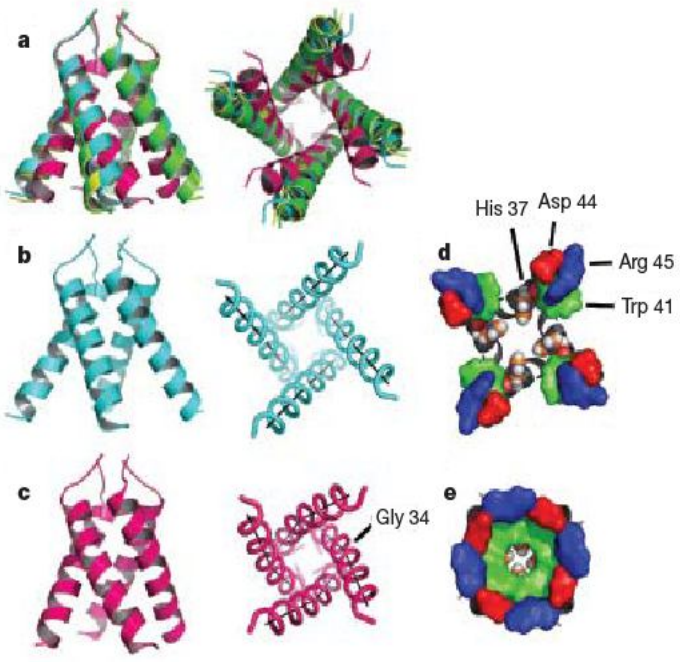

Figure 3 | Superpositions of the crystallographic tetramer demonstrate conformational differences in the C-terminal gating region of the channel. **a**, Side (left) and C-terminal (right) views of the crystal structure rotated and superimposed for all four cyclic permutations. **b**, The A4 bundle. **c**, The D4 bundle. **d**, **e**, The A4 (**d**) and D4 (**e**) bundles, viewed from inside the virus. The geometry was optimized by energy minimization using 1,000 cycles of XPLOR-NIH (see Methods), which resulted in only small changes in the backbone (0.8 Å and 1.4 Å r.m.s.d. for A4 and D4, respectively) and retained the native rotameric states of the side chains.

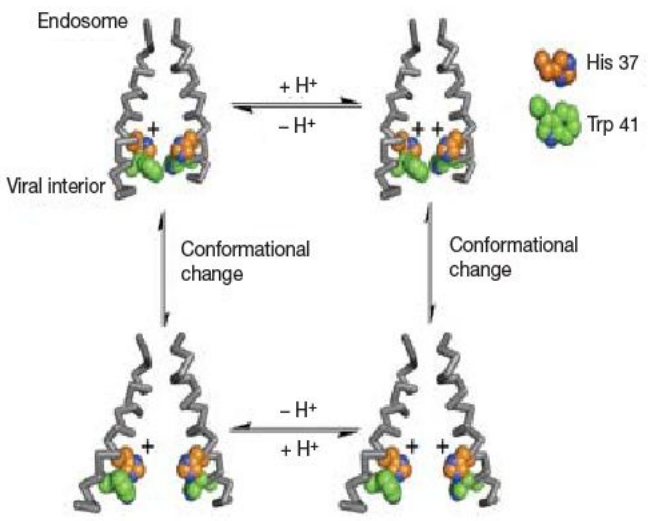

Figure 4 | Minimal mechanism of activation and conductance through the channel. Two helices of the tetramer and one protonation event are shown for simplicity.

mutants of M2. Inhibitors that target the cavity adjacent to the highly conserved His 37 and Trp 41 residues might reclaim the M2-blocking class of drugs both for prophylaxis and for treatment of ongoing endemic outbreaks and future pandemics of this deadly pathogen.

Note added in proof: An accompanying manuscript describes an NMR solution structure of a peptide comprising residues 18-60 of M2. We believe that this structure represents a biologically relevant closed form of the channel that is unable to bind amantadine in its central cavity[31].

METHODS SUMMARY

Crystals were grown by the hanging-drop method. X-ray data were phased by molecular replacement. Refinement methods and statistics are provided in the Methods; all residues were located in the most favourable region of the Ramachandran plot.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions D.S. grew the first high-resolution crystals of variants of M2TM, including the drug-free form reported here. A.L.S. selected additional variants and crystallized many other forms including the amantadine complex reported here. R.A. solved and refined both structures, and L.D.C. solved a monomeric form of M2TM used in molecular replacement. Data for the drug-free and amantadine-containing crystals were collected by S.S. and A.L.S., respectively. A.S.L. and V.T. also contributed to crystallization and data collection for crystal forms that were critical to obtaining and interpreting the crystal forms reported here. A.L.S., R.A., L.D.C., V.T. and S.S. processed and interpreted diffraction data. W.F.D., A.L.S., A.S.L., R.A., C.S.S. and V.N. each contributed to the analysis and understanding of the implications of the structure. C.S.S. and V.N. performed molecular modelling. W.F.D., A.S.L., A.L.S., R.A. and C.S.S. wrote the manuscript in consultation with the remaining authors. W.F.D. grew the first low-resolution crystals and supervised the project.

Author Information Coordinates for the apo form of M2TM have been deposited in the Protein Data Bank under the accession code 3BKD; coordinates for additional models are available from R.A. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/nature. Correspondence and requests for materials should be addressed to W.F.D. (wdegrado@mail.med.upenn.edu).

RETRACTION

doi:10.1038/nature06621

Anti-apoptotic function of a microRNA encoded by the HSV-1 latency-associated transcript

A. Gupta, J. J. Gartner, P. Sethupathy, A. G. Hatzigeorgiou & N. W. Fraser

Nature 442, 82–85 (2006)

We claimed that there was a microRNA in the exon 1 region of the latency-associated transcript of herpes simplex virus-1 (HSV-1) that downregulated the expression of transforming growth factor-β (TGF-β). We postulated that through this mechanism the virus prevented the latently infected cell from apoptosing. We are confident that HSV-1 downregulates *TGFB* messenger RNA; however, the mapping of this downregulation to the region of the HSV-1 genome that we predicted to encode the microRNA is not supported by further experiments in several laboratories.

B. R. Cullen from Duke University and D. Coen from Harvard University have not been able to detect the microRNA that we described. Using samples that they have supplied, we also been unable to consistently detect the predicted microRNA. Furthermore, in the samples supplied by the Cullen laboratory, we could only detect miR-LAT signal in SY5Y neuronal-like cell samples and not in non-neuronal 293 cell samples. The SY5Y cells were found to be positive for miR-LAT, regardless of whether or not they were infected with HSV-1, suggesting that we are detecting a SY5Y-encoded microRNA.

A.G. has declined to sign the retraction because she maintains that the original observations are correct.

An anti-body experience

What's in store for 2008?

Image supplied by Activ Motif

Antibodies are proven and valuable tools for scientists in various fields. For researchers, they are routinely used to identify and locate intracellular or extracellular proteins, as labelling agents for flow cytometry, or for immunoprecipitation experiments. Furthermore, antibodies have been exploited for many years for disease diagnostics and for developing therapeutics, with over 20 licensed mAb drugs on the market, some of which having reached blockbuster status. With their invaluable role in both research and medicine, there is much anticipation surrounding what might be achieved in 2008.

Antibodies for Research

Active Motif provides a variety of kits, assays, and primary and secondary antibodies for studying chromatin function and the biology of the nucleus.

The Company has recently added a new line of high-quality antibodies designed to analyse histones and histone modifications. These antibodies, many of which have been validated for use in chromatin immunoprecipitation (IP) and immunofluorescence, are ideal for studying the role of histones and histone modifications in the regulation of genome functions, from cell division and chromosome organisation to transcription and gene regulation. This complements Active Motif's already substantial line of transcription factor antibodies and antibody-based transcription factor activity assays. "Emerging techniques like chromatin IP require the use of high quality antibodies, and customers are demanding that antibody vendors supply products of suitable quality to meet these needs." said Jim Bone, Head of Strategic Marketing.

PBL InterferonSource, offers a range of neutralising antibodies raised against different interferons (IFNs) from various mammalian species. This provides a useful tool for researchers who study human interferon alpha on multiple cell lines to ascertain that the IFN-receptor interactions behave the same on each cell line. For identifying and sorting IFN-expressing cells in human and mouse, PBL also offers labelled antibodies with fluorescence dyes that include fluorescein isothiocyanate and phycoerythrin.

Antibody Production and Manufacture

Sartorius' CELLine is a disposable, two-compartment bioreactor for lab scale production of monoclonal antibodies and other secreted molecules. Efficient cell cultivation is dependent on optimal supply of oxygen and nutrients, as well as an efficient removal of growth-inhibiting metabolic waste products. In static cell culture the optimal balance of these factors is not achieved, leading to reduced cell growth and unfavourable conditions for achieving high levels of protein expression. The two-compartment bioreactor CELLine overcomes these limitations by dividing the bioreactor into a medium compartment and a cell compartment. A semi-permeable membrane between the compartments allows small molecules to diffuse from one compartment to the other.
Higher molecular weight molecules secreted by the proliferating cells are retained within the cell compartment. This results in a continuous flow of nutrients into the cell compartment and a concurrent removal of any inhibitory waste products.

The new 2-14 litre, **cGMP-compliant CelliGen® 310 bioreactor** from **New Brunswick Scientific** achieves extremely high yields of monoclonal antibodies and other proteins from a wide variety of mammalian, insect or plant cell lines. The compact benchtop system can be operated in batch, fed-batch or continuous modes, and is offered with five impeller options to enhance production from both anchorage-dependent and suspension cultures, with or without use of microcarriers. In perfusion mode, long-term production over the

The CELLine bioreactor from Sartorius

Sartorius Stedim Biotech's Vivapure® spin columns

course of 2 to 6 weeks can be sustained for high yields of up to 0.25-1.0 gram of protein/day, depending on impeller used. An advanced controller provides integrated control of system parameters as well as easy addition of multiple gas flow controllers, scales, analysers or other ancillary devices for optimising yields.

AbD Serotec's HuCAL® technology generates highly specific monoclonals in just 8 weeks, and offers many advantages over traditional animal immunisation approaches – including less restriction on antigen type, and the ability to engineer antibodies with peptide tags or enzyme activities for direct detection. HuCAL monoclonals are selected from a high-quality, synthetic library of more than 15 billion expressible antibody specificities.

Intelligent blocking and screening protocols mean that antibodies meeting demanding epitope specificity requirements can be quickly isolated and purified by HuCAL technology. Notable recent achievements include HuCAL monoclonals that can distinguish between phosphorylated and non-phosphorylated amino acid residues on very closely related peptides, or recognise oxidised amino acids in cell samples. Also, high affinity anti-idiotypic antibodies have been developed with a 100% success rate.

Purification and Characterisation

Sartorius Stedim Biotech has introduced **Vivapure® miniprep** and **maxiprep A & G** re-usable spin columns for fast and simple IgG purification. These chromatography resin-based spin columns combine the speed of centrifugal devices with the high capacities achievable with chromatography resin by the use of the patented FlowGo control technology. The FlowGo regulator ensures that the columns exert a back pressure, slowing down the sample flow in a centrifuge for maximal IgG binding to Protein A and G ligands. Vivapure miniprep and maxiprep A and G are optimally suited for antibody screening applications when parallel processing is needed. However, they are just as well suited for preparative IgG purifications.

The **Attana A100®** C-Fast and Attana´s new Rabbit Antibody Capturing Surface allow for screening and characterisation of rabbit polyclonal antibodies on a single chip, enabling researchers to find the best antibodies from their antisera without the need of purification. The use of the **Attana A100®** also provides detailed interaction kinetics and selection of antibodies, without the need for labels, resulting in faster and more cost effective research.

UNIchip® protein microarrays from **Protagen** provide a reproducible platform to determine specific binding profiles during antibody development in a quantitative way. The UNIchip protein biochips provide a novel and unique tool for antibody characterisation and ranking. Potential off-target activities can be detected by establishing a quantitative binding profile of an antibody to 400 different, unrelated targets. Together with on-chip determination of sensitivity, linearity and dynamic range the quantitative fingerprint enables a unique performance ranking and specificity analysis of antibodies. These features facilitate the timely analysis and comparison of antibody candidates, identifying those that are "fit for purpose" early in the development process, thus saving time and money. Currently, Protagen offers a range of four products in their UNIchip family, which are tailored to suit a specific application, depending on whether antibodies are targeted broadly, or more specifically against extracellular, intracellular or membrane proteins.

Labelling Reagents and Accessories

Invitrogen has launched new **Molecular Probes® Qdot®** nanocrystal conjugated primary antibodies for use in multicolour flow cytometry. These directly labelled fluorescent reagents allow cell biologists to use an array of distinct colours to track multiple parameters within a single experiment. When excited by a single light source, Qdot nanocrystals emit in a range of brilliant distinct colours with long-term photostability, offering researchers greater flexibility and precision in designing multicolour flow cytometry panels.

BioGenes' new **BlueCap Solutions** product range offers innovative buffer solutions for several uses in biotechnological, pharmaceutical and diagnostic research. These buffer solutions can be used for effective optimisation of immunoassays. The product line contains 14 products, including buffers with special features for improvement of results in ELISA, Western Blot and immunohistochemistry, as well as blocking and stabilisation solutions. The solutions will be produced by CANDOR Bioscience on behalf of BioGenes.

"Emerging techniques like chromatin IP require the use of high quality antibodies, and customers are demanding that antibody vendors supply products of suitable quality to meet these needs."

Jim Bone
Head of Strategic Marketing, Active Motif

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Faculty Position in Liver Research

The Division of Gastroenterology, Hepatology and Nutrition and the Liver Research Group of Cincinnati Children's Hospital Medical Center and Research Foundation invite applications for professorship, at either assistant or associate level, for research in liver regeneration or progenitor cell biology.

We seek a candidate with an MD or PhD degree and outstanding prospects for research, who will complement existing strengths of the Liver Research Group in the fields of liver development, regeneration and progenitor cells. A successful applicant must be committed to develop and implement independent, externally funded research programs, mentor students and facilitate interdisciplinary research. The successful applicant will be a member of a highly collaborative group of investigators and will have access to outstanding core resources at the NIH-funded Digestive Health Center and Cincinnati Children's Research Foundation.

Cincinnati Children's Hospital Medical Center and Research Foundation are internationally recognized as one of the nation's top pediatric care and research institutions. The Research Foundation ranks second nationally in NIH funding to full-service children's hospitals. Cincinnati is a friendly, pleasant, affordable city with a great quality of life, including many musical and theatrical programs, professional sports and nearby recreational opportunities.

Applicants should submit a curriculum vitae and statement of research interests to:
Dr. Jorge Bezerra, Director of Research
Division of Gastroenterology, Hepatology and Nutrition
Cincinnati Children's Hospital Medical Center
3333 Burnet Avenue, Cincinnati, OH 45229-3039
Jorge.Bezerra@cchmc.org

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NW123455R

THE CHINESE UNIVERSITY OF HONG KONG

Applications are invited for:-

Department of Biochemistry
(1) Research Assistant Professor

(Ref. 08/008(540)/2)

The Department has recognized protein science as a strategic research area and established the Centre for Protein Science and Crystallography. Further information about the Centre is available at *http://www.bch.cuhk.edu.hk/cpx*.

Applicants should have (i) a PhD degree; and (ii) an excellent track record of using protein crystallography and advanced biochemical techniques, preferably nuclear magnetic resonance spectroscopy for structure and function study of proteins with clinical significance. The appointee is expected to further enhance the research activities. Duties include (a) conducting research with the protein group of the Department on a strategic research project; (b) applying for competitive grants; and (c) undertaking some teaching. Appointment will initially be made on contract basis for two to three years commencing August 2008, renewable subject to performance, budget and mutual agreement. Applications will be accepted until the post is filled.

Department of Chemical Pathology
(2) Assistant Professor (Non-Clinical)

(Ref. 08/007(540)/2) (Closing date: February 29, 2008)

Applicants should have (i) a PhD degree in a relevant field; (ii) solid experience in bioinformatics research and knowledge of biostatistics; and (iii) a sound track record of publications and research accolades. The appointee will be responsible for providing input and expertise in the bioinformatics handling and interpretation of molecular genomic research data for developing innovative tests based on the analysis of genetic, epigenetic and RNA markers in blood plasma. Appointment will normally be made on contract basis for two years initially commencing as soon as possible, leading to longer-term appointment or substantiation later subject to mutual agreement.

Salary and Fringe Benefits

Salary will be highly competitive, commensurate with qualifications and experience. The University offers a comprehensive fringe benefit package, including medical care, and a contract-end gratuity for appointments of two years or longer, plus housing benefits for eligible appointees.

Further information about the University and the general terms of service for appointments is available at *http://www.cuhk.edu.hk/personnel*. The terms mentioned herein are for reference only and are subject to revision by the University.

Application Procedure

Please send full resume, copies of academic credentials, portfolio, a publication list and/or abstracts of selected published papers, together with names, addresses and fax numbers/e-mail addresses of three referees to whom applicants' consent has been given for their providing references (unless otherwise specified), to the Personnel Office, The Chinese University of Hong Kong, Shatin, Hong Kong (Fax: (852) 2603 6852) by the closing date. Please quote the reference number and mark 'Application - Confidential' on cover. The Personal Information Collection Statement will be provided upon request.

JP123618R

Physiology Faculty Positions

Applications are invited for Tenured or Tenure Track Faculty positions at any level from Assistant to Full Professor in the Department of Physiology at Emory University. We seek outstanding investigators to join a strong and established group devoted to basic cellular or molecular physiology and neuroscience. Areas of interest include membrane transport, ion channels and neuronal excitability, synaptic and cellular signaling mechanisms, and spinal cord function. We are particularly interested in candidates who can bridge these areas of interest. The successful candidate will be expected to establish an independent research program and participate in the scholarly activities of the Emory academic community. Women and minority candidates are encouraged to apply.

Applicants are asked to send by mail or electronically a curriculum vitae, a statement of research and teaching interests, and a list of 3-5 references to **Dr. Martin J. Pinter, Department of Physiology, Emory University, 615 Michael Street, Suite 648, Atlanta, GA 30322** or to **search@physio.emory.edu**. The review of applications will begin immediately and will continue until the available positions are filled.

Emory University is an Equal Opportunity/Affirmative Action Employer and Recruiter.

EMORY UNIVERSITY

NW123675R

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

Tenure-Track Assistant Professor of Cardiovascular or Neurosciences

VPA Reference #MEDI-2007-001

The Division of BioMedical Sciences in the Faculty of Medicine, Memorial University of Newfoundland invites applications for a tenure track position in cardiovascular or neurosciences at the Assistant Professor level. We seek applicants with research interests that compliment our existing areas of expertise in cardiovascular (www.med.mun.ca/basic/pages/programs_cardio.htm) or neuroscience research (www.med.mun.ca/basic/pages/programs_neuroscience.htm).

Applicants are required to have a Ph.D. and postdoctoral experience in cardiovascular sciences, neuroscience or a related field with clear evidence of research promise and scholarship. The successful candidate will be expected to develop an externally funded research program and participate in teaching at both the undergraduate and graduate level. In addition to teaching within their area of research interest, individuals with experience teaching Gross Anatomy would be of particular interest.

Further information about Memorial University and the Division of BioMedical Sciences can be found at www.mun.ca and www.med.mun.ca/basic. The Division also has research programs in Immunology/Infectious Diseases and Cancer/Developmental Biology, offering the potential for interdisciplinary collaborations. Interested applicants should submit a curriculum vitae, a detailed statement of research interests and goals and the names of three referees to **Dr. Karen Mearow, Professor and Associate Dean, Division of BioMedical Sciences, Faculty of Medicine, Health Sciences Centre, Memorial University of Newfoundland, St. John's, NL, Canada A1B 3V6 (jblundon@mun.ca)**.

Memorial University (www.mun.ca) is the largest university in Atlantic Canada. As the province's only University, Memorial plays an integral role in the education and cultural life of Newfoundland and Labrador. Offering diverse undergraduate and graduate programs to almost 18,000 students, the St. John's campus of Memorial offers a unique learning environment within the safe, friendly backdrop of the oldest city in North America. St. John's offers a vibrant cultural life with easy access to a wide range of year round outdoor activities.

Canadians and permanent residents of Canada will be given priority; however, all qualified candidates are encouraged to apply. Memorial University is committed to employment equity and encourages qualified applicants from women, men, visible minorities, aboriginal persons and persons with disabilities. The deadline for receipt of applications is **April 15, 2008** for a proposed start date of September 1, 2008.

NW123309R

naturejobs

On a recent trip to India, I saw first-hand just how difficult it can be to conduct scientific work and forge science policies in a rapidly developing nation. Even as India's scientific enterprise grows and matures, scientists must convince the government to provide increased funding in a country with a high number of impoverished citizens. Sound policies must wade through a system often mired in bureaucracy.

Take energy and the environment. Roughly half of India's 1.1 billion citizens have no electricity — instead millions of villagers use biomass and cattle-dung cakes to provide the energy for heating and cooking. Cities are polluted with particulate matter from diesel engines and coal-fired plants. Development threatens ecosystems and animal species such as the iconic tiger.

Meanwhile, India's economy craves energy to feed its impressive nearly double-digit growth in gross domestic product — leaving some worried about its growing carbon footprint. Use of alternative energy sources such as wind, biofuels and solar power is being expanded, but their contributions are dwarfed by demand. Even as the country churns out thousands of scientists and engineers, energy and environmental policies lie fallow — there is sometimes insufficient political will for them to become engines for change. To compound matters, the country's ministries often fight among themselves for power and leverage.

Bioscience, meanwhile, is booming in some parts of the country. In the private sector, Bangalore-based biotech giant Biocon has reported profits up 21%. Academia is showing signs of improvement too. Desirazu Rao, chair of the biochemistry department at Bangalore's Indian Institute of Science, lauds improved government funding and says more scientists are returning to India after doing postdocs abroad.

Indeed, human resources are key to India's success. If both industry and academia are to flourish, if there is any hope of ameliorating the energy and environment woes, India must retain and foster talent. Good science and science policies need a government that helps rather than hinders — they also need good scientists.

Gene Russo, acting editor of *Naturejobs*

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JOBS OF THE WEEK

Multiple Positions
Vertex
Cambridge, MA (USA)
Turn to page 8

Department of Zoology and Physiology Chair
University of Wyoming
Laramie, WY (USA)
Turn to page 28

12 Professorships
The Schleswig-Holstein Cluster of Excellence in Inflammation Research
Kiel (Germany)
Turn to page 14-15

Various Positions
Science Foundation Ireland
Various locations (Ireland)
Turn to page 2-3

Scientific Director
Leukaemia Research Fund
London (UK)
Turn to page 25

MOVERS

Rolf-Dieter Heuer, director-general, CERN, Geneva, Switzerland

2004–present: Research director, high-energy physics, DESY, Heidelberg, Germany
1998–2004: Professor, University of Hamburg, Hamburg, Germany
1994–98: Spokesman, OPAL Collaboration, CERN, Geneva, Switzerland

Rolf-Dieter Heuer built a strong career at the leading edge of particle physics, in part by listening to advice — for example, his high-school teacher confirmed his notion that a career in anything other than physics would be a mistake. As he prepares for his biggest career challenge yet — taking over in 2009 as director-general of CERN, the European laboratory for particle physics near Geneva — he plans to continue listening to his colleagues to better guide the future of international particle physics.

Heuer started off with a diploma in nuclear physics from the University of Stuttgart. But the detection of a subatomic particle, dubbed the J/ψ particle, intrigued him enough to alter his graduate plans. He subsequently worked on the neutral decays of the ψ' particle, the heavier sister of J/ψ, for his PhD at the University of Heidelberg. He was soon made a member of the JADE experiment (a collaboration between Japan, Germany and Britain) at DESY, the particle-physics centre in Heidelberg — one of the four experiments necessary to detect the gluon, the elementary particle that causes quarks to interact. "I was always motivated to work at the energy frontier — wherever that was," says Heuer. That frontier soon moved to the Omni-Purpose Apparatus (OPAL) at CERN's Large Electron–Positron collider, taking Heuer with it.

Heuer says his recipe for success as OPAL's spokesman was simple: give people enough freedom to do their job, which instils more motivation. After four years, Heuer moved to the University of Hamburg before becoming research director for the national particle-physics programme at DESY in 2004. There, he decided that working at the energy frontier meant expanding the energy frontier. So he focused the particle-physics efforts on CERN's Large Hadron Collider (LHC) and preparations for the International Linear Collider.

Heuer was an obvious choice to lead CERN, says former OPAL colleague Austin Ball. He says that as the LHC moves from construction to operation, it needs a physicist motivated by curiosity to pursue new science, including the Higgs boson, the missing piece of the standard model of particle physics, which is needed to explain how mass exists. Its detection is Heuer's top priority, as it will set the future of particle-physics research. Once that trajectory is set, Heuer must determine how to position CERN — taking into account preparations for the next colliders, decisions he doesn't take lightly. "Whatever CERN decides affects the worldwide community," he says. ■

Virginia Gewin

BRICKS & MORTAR

Singapore gears up for translation

Already a heavy-hitter in basic research, Singapore has set its sights on the clinic. Last month, the city-state opened its Institute of Medical Biology (IMB), dedicated to translating biomedical science into treatments, in the Biopolis campus.

Biopolis, a 18.5-hectare complex, opened six years ago and is still under construction. More than 1,000 scientists study molecular biology, bioinformatics and bioengineering in its seven institutes. The IMB's 37,000 square metres houses 120 scientists in 13 labs. Researchers started moving in last April.

At the IMB, applying science to the clinic — translational research — comes first, says director Birgit Lane. "People aren't finding they have to pay lip service to translational principles in order to get their funding," she says.

Lane wants her colleagues to strike up collaborations with physicians in Singapore and abroad. A cell biologist who studies rare skin disorders, Lane works with doctors at Singapore's National Skin Centre, which provides her with samples from patients.

And IMB researcher Sai Kiang Lim also works in Singapore National University's surgery department. Her team uses mesenchymal stem cells derived from embryonic stem cells as a treatment for heart disease. At the moment, she is using cell and animal models, but clinical studies are on the horizon.

"I have always been collaborating with clinicians but we were never able to bring anything close to the clinic," says Lim. If all goes well at the IMB, the clinic should not be far off, she says.

Lim keeps her clinical collaborators up to date on her research so they can anticipate stumbling blocks. She also directs her bench work towards treatments: her lab tests therapies on diabetic pigs, which are a better stand-in for humans with heart disease than healthy animals are.

"We can already see a big difference in the amount of clinical material we're going to have from the Singapore site," says Irwin McClean, a geneticist at the University of Dundee in Scotland who collaborates with Lane.

Singapore's biomedical boom has sucked up talent from around the world, and Lane says the IMB plans to hire up to seven more investigators.

Yet despite bold goals, the IMB already faces a hurdle: two of its most prominent scientists now split their time with Britain. Stem-cell pioneer Alan Colman is about to join King's College London. And from this month, Lane will lead a biomedical centre at the University of Dundee. ■

Ewen Callaway

POSTDOC JOURNAL

Biopolis dreams

The Biopolis complex in Singapore is both an inspiring and daunting place to do a postdoc. The motto for the complex of nine glass buildings erected within the past seven years is "racing with the world's best towards the very limits of modern science". These ambitions clash spectacularly with the mundane realities of lab work. As a postdoctoral fellow there, I am acutely aware of this stark contrast between dream and reality. It weighs heavily on me as I attempt to chart my course through a new year fraught with uncertainty.

Furthermore, how do I adapt to the local working environment? Research is not done in a vacuum — everyone makes a difference, whether administrative staff, a lab technician or the 'big boss'. Having been educated in the United States, I notice many differences, such as in communication style, approach to project management and work ethic. The highly structured and hierarchical nature of an Asian workplace contrasts with the collegial atmosphere of a US academic institution, and personal interactions differ accordingly. Figuring out how to respond and adapt is important in our international community.

The new year will bring many changes and promises to be challenging at many different levels. I have to remain focused and positive, and hope to emerge a better scientist and person. ■

Amanda Goh is a postdoctoral fellow in cell biology under the Agency of Science, Technology and Research in Singapore.

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SFI has a flexible grants and awards portfolio and several times a year issues calls for proposals from scientists and engineers. SFI also accepts unsolicited proposals throughout the year. SFI's award programmes include:

Principal Investigator Programme

for outstanding researchers, normally ranging between €50,000 - €1 million per year and may be up to five years in duration.

Research Professor Recruitment Awards

for outstanding researchers, with particularly distinguished international reputations, awards normally ranging up to €500,000 per annum for up to two years.

E.T.S. Walton Visitor Awards

supporting leading international scientists who visit Ireland to undertake research for up to one year, normally ranging up to €200,000.

President of Ireland Young Research Awards (PIYRA)

attracting to Ireland and supporting Irish researchers within five years of completing their PhD, normally up to €1 million over five years.

Trinity College Dublin

Further Details:
www.tcd.ie/vacancies

- Postgraduate Studentships (Ph.D.) in Protein X-ray Crystallography (Biochemistry and Immunology)
- Postdoctoral Research Fellows positions in Protein X-ray Crystallography (Biochemistry and Immunology)
- Postdoctoral Research Fellow to work on the Physics and Engineering aspects of Biaxial Nematic Devices (School of Engineering)
- Ph.D. Studentship in the Physics and Engineering aspects of Biaxial Nematic Devices (School of Engineering)
- Postdoctoral position in Immunology Research (School of Medicine)
- Ph.D. Studentships (2) in Computational Materials Theory (School of Physics)

Immunology Research Centre

This is an academic-Industry partnership lead by Trinity College Dublin (TCD) in collaboration with NUI Maynooth (NUIM). Applications should be directed to the relevant university indicated by the name in brackets.

TCD: www.tcd.ie/vacancies
NUIM: http://personnel.nuim.ie/vacancies.shtml

- Senior Scientist Position (Fixed term contract) to manage €10m research programme on discovery and function of exogenous and endogenous regulators of innate immunity (TCD)
- Postdoctoral position (x2) in discovery of endogenous activators of innate immunity (TCD)
- Postdoctoral position (x3) in discovery of bacteria and parasite-derived regulators of innate immunity (TCD)
- Postdoctoral position (x2) in signalling pathways induced by novel agonists of TLR and NLRs (TCD)
- Postdoctoral position in viral modulators of innate immune signalling (TCD)
- Postdoctoral position in endogenous regulators of innate immune signalling (NUIM)
- Postdoctoral position in NK cell activation by endogenous / exogenous regulators (TCD)
- Postdoctoral position in innate driving adaptive immunity (TCD)
- Postdoctoral position in regulators of innate immunity in models of autoimmunity (TCD)
- PhD Fellowship in endogenous regulators of innate immunity (TCD)
- PhD Fellowship in signalling pathways induced by novel agonists of TLR and NLRs (TCD)
- PhD Fellowship in function of novel TLR and NLR agonists in models of cancer (TCD)
- PhD Fellowship in dendritic cell activation by endogenous / exogenous regulators (TCD)
- PhD Fellowship in viral-derived activators of RLR and DNAR (TCD)

University College Dublin

Further Details:
www.ucd.ie/jobopportunities

UCD Conway Institute of Biomolecular and Biomedical Research

Bioinformatician (Research Fellow) for Next-generation sequencing data management and analysis

UCD BioNanoInteract SRC

Strategic Research Centre Manager

Systems and Data Manager

Claude Shannon Institute

Post Doctoral Fellowships: Coding Theory, Cryptography, Discrete Mathematics, Algebra, Algebraic Geometry, Number theory

Dublin City University

Further Details:
www.dcu.ie/vacancies/current.shtml

PhD Scholarships in Dielectric materials / Wide bandgap oxide nanostructures

PhD Scholarship Electrohydrodynamic Focusing in 2-Dimensional Planar Microfluidic Devices

National Centre for Plasma Science and Technology (NCPST)

PhD Scholarship in Plasma Physics

Centre for Next Generation Localisation (NGL)

This is a multi-university collaborative initiative lead by DCU. Applications should be addressed directly to the relevant University, indicated in brackets:
UCD: www.ucd.ie
TCD: www.tcd.ie/vacancies
DCU: www.dcu.ie/vacancies/current.shtml
UL: www.ul.ie/vacancies

Language Technology

Postdoctoral Positions in Machine Translation (DCU)

Postdoctoral Position in Natural Language Processing (DCU)

PhD Scholarships in Machine Translation (DCU)

Postdoctoral Position in Speech Processing (UCD)

PhD Scholarships in Modelling Phonetic Parameters & Voice Characteristics (UCD)

PhD Scholarships in Speech Synthesis (UCD)

PhD Scholarships in Speech Recognition (UCD)

Postdoctoral Position in Natural Language Processing (TCD)

PhD Scholarships in Modelling Phonetic Parameters & Voice Characteristics (TCD)

PhD Scholarships in Natural Language Processing (TCD)

Digital Content Management

Postdoctoral Positions in Web Search and Web Information Retrieval (TCD)

Postdoctoral Position in Adaptive Hypermedia and Adaptive Web Services (TCD)

PhD Scholarships in User Modelling and Personalisation (TCD)

PhD Scholarships in Ontology Modelling and Semantic Web Technologies (TCD)

PhD Scholarships in Web Content Harvesting and Semantic Model Generation (TCD)

PhD Scholarships in Dynamic Adaptive Web Composition for Multilingual Digital Content (TCD)

PhD Scholarships in Dynamic Personalisation of Web Services (TCD)

System Framework

Postdoctoral Position in Human Factors for Natural Language Processing (TCD)

Postdoctoral Position in Interaction Design for Natural Language Applications (TCD)

Postdoctoral Positions in Software Engineering for Natural Language Processing (TCD)

PhD Scholarship in Multilingual Speech and Text Interface Design (TCD)

PhD Scholarship in User, Context and Work Analysis of Natural Language Applications (TCD)

PhD Scholarships in Web Service Integration (TCD)

Localisation

Postdoctoral Position in Multilingual Digital Content Development (UL)

Postdoctoral Position in Digital Content Translation and Adaptation Technologies (UL)

Postdoctoral Position in Localisation Process and Workflow Automation (UL)

PhD Scholarships in Multilingual Digital Content Development (UL)

PhD Scholarships in Digital Content Translation and Adaptation Technologies (UL)

PhD Scholarships in Localisation Process and Workflow Automation (UL)

Biomedical Diagnostics Institute (BDI)

Research Nurse: Cardiology

Postdoctoral positions in Advanced Biosensor Development/ Microfluidics/ Fluorescence Enhancement – Optical Instrumentation

Technician positions in Analytical Instrumentation / High-Throughput Antibody Screening / Optical Instrumentation
PhD Scholarship in Plasmonic Enhancement of Fluorescence

Athlone Institute of Technology

Further Details:
http://www.ait.ie/vacancies/

PhD in Biomedical Microbiology.

University of Limerick

Further Details:
www.ul.ie/hrvacancies

PhD Studentship in Engineering - Sensor Systems

Solid State Pharmaceutical Cluster

This is a multi-university collaborative initiative led by UL. Applications should be addressed directly to the relevant University, indicated in brackets:
UCC: http://hr.ucc.ie/EmploymentOpportunities/Academic Vacancies
UCD: www.ucd.ie
TCD:www.tcd.ie/vacancies
NUIG: www.nuigalway.ie/vacancies
UL: www.ul.ie/vacancies

Postdoctoral Researcher: In-situ analysis of crystallisation of pharmaceutical solids (UL)

Research Facilitator (UL)

Postdoctoral Researcher: Chemometrics and Analytical Chemistry (NUIG)

Postdoctoral Researcher: Organic Chemistry, crystal engineering (UCC)

Postdoctoral Researcher: Chemical Engineering of Pharmaceutical Processes (UCD)

Postdoctoral Researcher: Pharmaceutics or a related discipline with experience of physiochemical characeterisation and amorphous material (TCD)

University College Cork

Further Details: **http://hr.ucc.ie/EmploymentOpportunities/AcademicVacancies**

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W123666R

Grow your career with us.

NIBRT was established by the Government and IDA to promote Research and Training in the area of Bio-processing technology. **NIBRT** is an innovative collaboration between four of Ireland's Higher Education Institutes, University College Dublin (UCD), Trinity College Dublin, Dublin City University, Sligo Institute of Technology and other third-level and industry partners.

The National Institute is currently based on campus in UCD where it is intended to establish a specialised facility which will deliver world class research and training. It will include a bio-processing complex which will allow training of students and graduates to obtain real-time experience in a pilot plant environment.

We are currently seeking to recruit for a number of key posts working with **Professor Pauline Rudd** and her international scientific team, the Dublin-Oxford Glycobiology Laboratory, currently based in the Conway Institute in UCD. Each position is well supported by the existing team and full training is available in the technologies described.

Senior Post Doctoral Research Fellow - Mass Spectrometry

Ref 001/08

The successful candidate will become a world leader in glycan analysis mass spec technologies by building on the existing expertise in the Dublin-Oxford Glycobiology Laboratory to develop new strategies for N- and O-glycan analysis. The person appointed will support the contract and bioprocessing research of the group, assist with various glycoproteomics initiatives, provide lectures on mass spectrometry for glycobiology training courses and prepare reports, papers, grant proposals and presentations.

Senior Post Doctoral Research Fellow Molecular Biology

Ref 002/08

The successful candidate will continue a collaborative bio-processing project to manipulate the glycosylation of IgG based on an understanding of cellular pathways and IgG structure. The aim is to understand roles for IgG glycosylation in effector functions to improve the efficacy and safety of pharmaceutical compounds. You will be responsible for report writing, grant writing and review of publications and staff supervision.

Postdoctoral Research Assistant

Ref 003/08

The successful candidate will support the Dublin-Oxford Laboratory initiatives that involve molecular biology. In particular, you will assist the Senior Postdoctoral Research Fellow in modifying the glycosylation of IgG and investigating novel ways to manipulate cellular glycosylation pathways.

Senior Post Doctoral Research Fellow *Collaboration with Eli Lilly and Co.*

Ref 004/08

The successful candidate will lead on a collaborative research programme with Eli Lilly. The aim is to interface the technology platforms developed in the Dublin-Oxford Laboratory directly with a bioreactor to monitor both N- and O-linked glycosylation in biopharmaceutical production lines and to transfer technology to Lilly bases in the US and in Ireland. The techniques will involve detailed HPLC and MS based glycan analysis and the use of molecular biology approaches to modulate glycosylation.

Senior Technician *Collaboration with Eli Lilly and Co.*

Ref 005/08

The successful candidate will work with the Senior Post Doctoral Research Fellow to transfer technology to Eli Lilly and Co. and to work on proprietary data bases generated in this programme. In addition, you will assist in training Lilly employees in glycobiology techniques and enable the transfer of technology to Lilly bases in the US and Ireland.

Post Doctoral Research Assistant

Ref 006/08

Reproductive biology strategic research cluster to investigate fertility in cattle.

The successful candidate will work in an interdisciplinary team and with members of the Dublin-Oxford Laboratory to build data bases and improve technology for automated O-glycan analysis, determine the variation of N- and O-glycosylation of mucins with the oestrus cycle, develop an understanding of the biology and potential functions of glycans on mucins and of the glycosylation pathways that control them and when appropriate, initiate the development of an analytical kit.

Post Doctoral Research Assistant *Breast Cancer Ireland Research Grant*

Ref 007/08

The successful candidate will join an existing team to identify and validate a panel of potential glycosylated biomarkers for breast and lung cancer. The principle technologies involved include HPLC and MS based glycan analysis and glycoproteomics. Molecular biology skills will also be required to investigate the pathways responsible for the glycosylation changes in disease.

Further details on the posts and job descriptions can be found on **www.nibrt.ie** or for an informal discussion regarding the post please contact **Siobhan Browne, HR Advisor** on **00 353 86 826 3957** or email **Siobhan.browne@nibrt.ie**

Applications can be submitted including all relevant documentation and quoting reference number to:
Siobhan Browne, NIBRT, Engineering Building, UCD, Belfield, Dublin 4, Ireland.
Or by email to siobhan.browne@nibrt.ie

We offer highly competitive packages to successful applicants.

The closing date for receipt of applications is Tuesday, 21st February 2008 by 5pm.
NIBRT is an equal opportunities employer.

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National Institute for Bioprocessing Research and Training

School of Health & Bioscience

LECTURER/SENIOR LECTURER IN MICROBIOLOGY/BIOTECHNOLOGY

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Salary in the range
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The University of East London is a dynamic and rapidly expanding University in the heart of Europe's largest regeneration area and immediately adjacent to the site of the 2012 Olympics. It is a vibrant and exciting environment in which to work.

Based at our Stratford campus, our School brings together UEL's innovative research and teaching in the fields of Biosciences, Health Studies and Professional Health Sciences. Our key research themes are Bioscience, Sports Science, Infection and Immunity, Complementary Medicine, Rehabilitation and Public Health and we are committed to expanding our research profile to reflect the multi-disciplinary nature and diversity of approach within the School. We have a successful and expanding portfolio of taught programmes and exciting new postgraduate programmes being developed. These programmes are a key element in the school strategy and the opportunities opened up through the Olympics and Paralympics are considerable.

We are looking for a new colleague who shares our creativity and energy and our passion for equality and diversity to help us promote our reputation and realise our vision in relation to our Olympic activities and contribute to the School's, research, teaching and administrative activities in the area of Microbiology/Biotechnology.

You will have a degree, a PhD in a relevant area and a proven research record. Expertise in student project supervision is advantageous. Please note that you will be required to work occasional evenings and weekends.

To obtain further details about this vacancy please visit our website at: www.uel.ac.uk/vacancies or e-mail: recruitment@uel.ac.uk quoting reference number 13a2008. The closing date for applications is 14 February 2008. CVs without completed application forms will not be accepted.

www.uel.ac.uk

We are working actively to improve the diversity of our staff.

U123877RM

HOCHSCHULE BIBERACH

BIBERACH UNIVERSITY OF APPLIED SCIENCES

The Faculty of Pharmaceutical Biotechnology at the University of Applied Sciences Biberach in Baden-Württemberg, Germany invites applications for two professorships for September, 2008.

W2- Professorship
(Reference number PBT 08)

for development and control of biotechnological production processes. In the first instance this position is planned until December, 2015. This appointment can also be fullfilled as a part time position.

The outstanding candidate should have a Ph.D. and extensive experience (an extensive track record) in current bioprocess technologies involving eukaryotic cell cultures. A good knowledge of the German language is essential.

The successful applicant will be expected to give lectures and lead practical exercises in cell culture technology, development of biotechnological processes and related disciplines.

Further information about the curriculum and the complete job advertisement in German are available from Prof. Dr. Hannemann, Fon 00 49-(0)73 51/5 82-4 50.

W2-Professorship part time
(Reference number PBT 09)

for pharmacology and pharmaceutical technology. This part-time position (50 %) is in the first instance planned for a period of five years.

We are looking for an applicant with an above-average university degree and a Ph.D. Several years experience in pharmacy, pharmaceutical technologies and a good knowledge of the German language are essential.

The future job holder is expected to give lectures and to lead practical exercises in pharmacy, pharmaceutical technology, pharmacology, toxicology, drug- and biotechnology-related legislation and similar disciplines.

Both professorships are part of the new course on Pharmaceutical Biotechnology in the faculty with the same name.

The requirements of employment arise from the "Landeshochschulgesetz Baden-Württemberg". More information and the complete job advertisement in German are available at **http://www.fh-biberach.de/service/Stellenanzeigen**.

Applications with the usual documents including the **reference number PBT 08 or PBT 09** are requested before **February 29th, 2008** to the following address: **Personalabteilung der Hochschule Biberach, Karlstr. 11, 88400 Biberach, Germany, Fon 00 49-(0) 73 51/5 82-1 20.**

W123665R

Start your own research team within the challenging environment of VIB

VIB, the Flanders Institute for **Biotechnology**, is an **entrepreneurial** research institute in Flanders, Belgium where 1.000 scientists and technical staff conduct basic research in a number of life science domains. The annual research budget is about 70 M€. The scientific mission of the institute is to significantly push the frontline of life sciences by frequent scientific breakthroughs and highly significant contributions. During the past years we have created an environment that stimulates talent and excellence. The major ingredients needed to provide such environment are a triggering critical mass of exciting scientists, stimulating discussion, long-term and stable financing, state of the art infrastructure and access to advanced central core technologies. VIB pursues an active patent and licensing policy with the objective to translate research results into products for the industry and the public at large. VIB also develops educational material and provides information about life sciences to the public.

VIB

Independent principal investigators

VIB is seeking to appoint up to 5 outstanding early stage independent investigators working in emerging and interdisciplinary areas within or related to the life sciences. These include but are not limited to systems biology, molecular imaging, computational and mathematical biology, small animal and cell-based model systems, whole-genome analysis and translational research.
The investigators will be appointed as independent Group Leaders with the option to be integrated into suitable VIB Departments. They will be funded with a VIB grant of 2.0 M€, to be spent in a 5-year period and renewable on a competitive basis.

Your opportunity

Eligible applicants have several years of postdoctoral research experience and have a proven track record, comprising several publications in high impact journals. Applicants are also capable of skilfully managing a research team. Applicants are expected to perform research of the highest international standard to be published in the best journals in the field. The results obtained through this research ideally have the potential for industrial applicability.

For each group VIB will provide an overall grant of about 2 Million Euro, covering a 4-5 period. This grant is dedicated to start up and can be used flexibly to support the salary of the group leader, a small team (4-5 people), induding postdoctoral and/or postgraduate researchers as well as dedicated technical support, lab equipment and consumables. The candidate is expected to complement this budget through other national and international grants and through industrial collaborations.

How to apply and time line

Full details of the call and guidance on submitting your application are available at **www.vib.be/jobs.** Proposals should be submitted electronically, before 31st March 2008 to **lieve.ongena@vib.be.** A short list of applications will be selected on the basis of CV and research program proposal. The corresponding applicants will be invited for interviews and to give a seminar in VIB mid 2008. Final selection of the new projects is scheduled in the summer of 2008. Selected candidates are expected to start their group in VIB within a year after selection.

How to apply?

For more information on VIB, please consult our annual reports and website (www.vib.be) or contact Lieve Ongena, science policy manager at VIB.

e-mail: lieve.ongena@vib.be - tel. +32 9 244 66 11

www.vib.be

W123066R

Ortho Biotech Oncology Research and Development (ORD) is a Division of Janssen Pharmaceutica N.V., and has a major commitment in bringing novel and innovative new cancer drugs to the clinic. The Beerse site in Belgium is a key driver of this initiative, having taken three novel targeted small molecule anti-cancer agents into the clinic over the last two years, and with two new-targeted agents positioned to enter the clinic during 2008.

To enhance its leadership position and focus for the future, ORD is now deploying a new strategic approach in Oncology, focusing on future leadership in scientific insights and understanding of cancer as a disease of malignant tissues. We are looking at areas including migration, invasion, epithelial-mesenchyme transition, and nutrient sensor pathways.

We are now seeking to recruit a number of scientists to provide important research insights into these areas and to engage on new drug discovery and biomarker projects to impact our Oncology pipeline.

Positions in Cancer Drug Discovery, Research and Biomarkers

(M/F) Ref. 072112/Nature

Cancer Drug Discovery positions
Tenured posts are available for post-doctoral and graduate level scientists to expand our Cancer Biology Department. Oncology-related experience in molecular/cell biology, signal transduction pathways and assay development are required. The ability to work in multi-disciplinary Teams during the initiation and maturation of the drug discovery Projects will also be very important.

Cancer Research positions
Several 3-year post-doctoral positions are available for Ph.D. levels scientists to work on cutting-edge research aimed at characterizing novel signaling pathways and putative targets in the areas of cancer cell migration/invasion, and epithelial-mesenchyme transition. Preference may be given to candidates with experience in these areas. These studies will be highly interactive with external research centers of excellence, and USA Ortho Biotech Oncology colleagues. It is expected that publications will result from these studies.

Biomarker positions
Tenured posts are available for post-doctoral and graduate level scientists to expand our studies in this area. There is a major commitment to biomarkers within Ortho Biotech Oncology, and all of our Oncology Projects have substantial integrated biomarker programmes from lead optimization, through to all stages of clinical trials. A senior team leader position is available for candidates experienced in biomarker research (pharmacodynamic and predictive response markers). Positions are also available for the analysis of microarray data to derive biomarkers requiring an excellent understanding of statistics. Additional lab based positions supporting the discovery of pharmacodynamic and predictive markers are available.

All of the positions will be based at the excellent Oncology research facilities located on the Beerse site in Belgium (located close to Antwerp). Since this is an extensive multi-national research site, English is the spoken & written language everywhere, so it is important that applicants have good mastery of English. These positions offer excellent career opportunities in an environment where there is highly active on-going drug discovery, translational research and clinical studies for cancer.

Janssen Pharmaceutica is the largest pharmaceutical company in Belgium. We are also the largest affiliate of Johnson & Johnson, the world leader in healthcare.

Janssen Pharmaceutica is synonymous with ground-breaking research. The results speak for themselves: in Beerse more than 80 innovative drugs have already been discovered.

Motivated professionals find rich development opportunities and multiple challenges at Janssen Pharmaceutica. They are given every opportunity to grow.

JANSSEN PHARMACEUTICA

W123757R

Interested applicants should apply by sending a copy of their CV in English (by preference via e-mail), to Kristine Deckx (kdeckx@janbe.jnj.com), Human Resources Department, Janssen Pharmaceutica N.V. (a Division of Johnson & Johnson Pharmaceuticals), Beerse, Belgium. For more information, please check the website www.janssenpharmaceutica.be/jobs or contact Ellen Driesen at 00 32 14602309.

ORTHO BIOTECH ONCOLOGY RESEARCH & DEVELOPMENT
DIVISION OF JANSSEN PHARMACEUTICA N.V.

progress through innovation

Medicines for Malaria Venture

Medicines for Malaria Venture (MMV)

SIXTH CALL FOR LETTERS OF INTEREST

Medicines for Malaria Venture is a not-for-profit Organization committed to the discovery, development and delivery of affordable anti-malarial drugs through public-private partnerships. We currently have four new medicines in late stage clinical development. We are now looking to the next generation of medicines. Proposals for new and innovative projects are sought both for Discovery and Clinical Development.

Discovery: Projects should either have a well defined molecular target, or involve screening against parasites in vitro. In vivo screens should focus on specific aspects of the lifecycle beyond simple life/death screens where possible. We particularly welcome proposals for studying P. vivax liver stage infection. The malaria community is now focusing efforts on the long term elimination of the disease, and proposals should reflect how they can be specifically adapted to this goal.

Clinical Development: Projects can be new medicines or new treatment regimens, with particular focus on children, pregnant women and/or Intermittent Preventative Treatment (IPT). We particularly welcome proposals that specifically focus on targets for long term elimination of malaria.

Applications may be from single institutions or partnerships between an academic centre and a Pharmaceutical company.

The initial application should be by sending a letter of interest of no more than three pages to:
Dr. Ian Bathurst, MMV, Rte de Pré-Bois, 20
P.O.Box 1826, CH-1215 Geneva 15, Switzerland

E-mail: **applications@mmv.org**

Applications should reach us by March 15th 2008. Electronic submissions are preferred.

More details of the call can be found at **www.mmv.org**

MMV gratefully acknowledges the funding and support it has received from: Bill and Melinda Gates Foundation, BHP Billiton, ExxonMobil Corporation, Global Forum for Health Research, International Federation of Pharmaceutical Manufacturers Associations, Irish Aid, The Netherlands Ministry for Developmental Cooperation, NIH, Rockefeller Foundation, Roll Back Malaria Partnership, Swiss Agency for Development and Cooperation, United Kingdom Department for International Development, United States Agency for International Development, World Bank, World Health Organization, WHO/TDR, Wellcome Trust.

W123640A

JOHANN WOLFGANG GOETHE
UNIVERSITÄT
FRANKFURT AM MAIN

The Center for Drug Research, Development and Safety (ZAFES) of the University of Frankfurt, Germany, invites applications for an

Independent Junior Research Group Leader

(selbständige Nachwuchsgruppe)
in the field of
Lipid Signaling

within the newly established Lipid Signaling Center Frankfurt (LiFF). The research group will be equipped with the position of the group leader, a post-doc, a technician and an annual budget for consumables.

The Center for Drug Research, Development and Safety (ZAFES) is part of the University of Frankfurt and has more than 30 member institutes comprising a broad spectrum of expertise and technology in medical research and life sciences. It provides state-of-the-art facilities for molecular biology, cell biology and clinical investigations. The LiFF, as a joint research program within the ZAFES, unites various aspects of research on lipid signaling. Particular foci are the signaling cascades of sphingolipids, arachidonic acid and endocannabinoids and the physiological, pathophysiological and pharmacological consequences in cardiovascular, oncological, and neurological disorders.

Candidates are expected to have a Ph.D., M.D. or equivalent degree, a strong track record of internationally recognized research in the field of lipid signaling and the potential to establish an independent research program with a focus on arachidonic acid cascade, endocannabinoids or sphingolipids in order to complement the existing research groups. It is expected that the successful candidate will interact with other groups in the center.

The initial appointment is for three years with the possibility of renewal for an additional three-year period and promotion to a permanent position. Salary and social benefits will be in accordance with the University regulations for academic personnel.

The University of Frankfurt has a policy of raising the proportion of women in academic positions and therefore encourages applications from women with the necessary qualification. Under German law disabled applicants with full qualifications will be treated preferentially.

Qualified persons interested to work at the LiFF and having relevant expertise are invited to send their application by mail or e-mail to **The Director of the Institute of Clinical Pharmacology, Prof. Dr. Dr. Gerd Geisslinger, ZAFES/ Institut für Klinische Pharmakologie, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany (Geisslinger@em.uni-frankfurt.de).**

www.uni-frankfurt.de

W123761R

Obra Social
Fundación "la Caixa"

CNB
CENTRO NACIONAL DE BIOTECNOLOGÍA

The Spanish National Centre of Biotechnology (CNB-CSIC) in Madrid, Spain invites applications for

10 PhD Fellowships

in collaboration with Fundación La Caixa.

Fellowships are available for a period of 48 months, starting in September-October 2008.

The CNB is dedicated to improving human and animal health, as well as solving environmental and agricultural challenges. The excellence of research conducted ranks the CNB among the leading research institutions in the world (as evaluated by EMBO in 2005).

The major areas of study are:

- Macromolecular Structures
- Molecular and Cellular Biology
- Microbial Biotechnology
- Plant Molecular Genetics
- Immunology and Oncology

Highly qualified and motivated applicants from all over the world are invited to submit their application before March 31st 2008.
See our website (http://www.cnb.csic.es) for a list of PhD projects available and for additional information and application procedure.

W123321R

Novartis Vaccines

For all the right reasons...

Working at Novartis Vaccines Italy means being part of a dynamic and stimulating environment where each associate can express to the fullest his or her professional and personal capabilities within the different company sectors of the Siena and Rosia locations. Our company, in fact, is an integrated company where the product pipeline ranges from research and development to manufacturing and marketing of the vaccines.
In particular, the Novartis Vaccines Research Centre is a jewel in the crown of Italian and international Research, thanks to the 160 researchers who come from all over the world to work in the Siena laboratories.
The future of Novartis Vaccines Research is even more promising thanks to its numerous partnerships with national and international Organizations.

Novartis Vaccines is a multinational company striving for excellence and innovation. In order to successfully reach our goals, we are constantly looking for resourceful and enthusiastic professionals capable of taking on the responsibilities required by their position within the company and who are also passionate about contributing to the development and sharing of new ideas. If you want to join us, visit our Career section in the website www.novartisvaccines.com!

www.novartisvaccines.com
Don't forget to mention naturejobs when applying via website.

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Epic
Investing in New Therapies

5th Annual EPIC - European Partnering and Investment Conference

Sponsored by

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May 22 2008 Cumberland Hotel, London

EPIC has now become a favourite fixture on the biotechnology conference circuit with a reputation for high value for a low entry cost. The formula of industry keynotes, a critical mass of over 60 UK and European biotechnology company presentations, plus all day partnering has proved to be simple yet successful. The one day event hosts some 300 delegates, showcasing emerging, small and medium sized UK and European biotechnology companies and incorporating new spin outs each year.

It is the perfect opportunity to hear from and meet a range of growing biotechnology companies who need a combination of partners, investment and professional services to develop their companies. Two of the streams this year focus on companies with products in either pre-clinical or clinical trial stages. These will be of particular interest to pharmaceutical and biotechnology companies and CROs. Having over 60 biotechnology companies presenting in one day makes EPIC a cost effective way of evaluating and meeting companies. The very popular University Challenge session again features along with two sessions for Platform and Diagnostics companies

For on-line registration and programme details please go to www.epicbiotech.com

For sponsorship and presenter enquiries please contact EPIC Director, Simon Tarpey at simontarpey@epicbiotech.com 00353 87 1386419

For conference administration enquiries please contact Debbie at Ya Ya Events on 44 207 989 2424 or Debbie@ya-ya.co.uk

Principal sponsors Merck Sharp & Dohme Limited (MSD) is the UK subsidiary of Merck & Co., Inc., of Whitehouse Station, New Jersey, USA, a leading research-based pharmaceutical company that discovers, develops, manufactures and markets a wide range of innovative pharmaceutical products to improve human health.

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BIOBASE
BIOLOGICAL DATABASES

BIOBASE, headquartered in Wolfenbüttel, Germany, is a leading provider of biological databases, knowledge tools, software and services for the life science industry, with branch offices in the USA, India and Japan.

BIOBASE provides an excellent scientific environment and actively participates in international research projects on developing systems biology tools and revealing molecular mechanisms of human diseases, drug target and biomarker discovery. In the framework of these research projects, funded by European Committee and German Ministry of Science and Education, we have an immediate opening of several scientific positions in our German office.

Bioinformatics/Systems Biology Scientist ***Job Code: BI-01***

To lead studies in systems biology, developing tools for the analysis and modeling of molecular mechanisms of diseases (such as cancer, diabetes, neurodegeneration, infection).

- PhD in Bioinformatics, Physics, Computer Science Molecular Biology, Genetics, or in other life sciences.
- Strong skills in programming, mathematics, statistics; experience in modeling and simulations.
- Knowledge and interest in Molecular Biology, Genetics, Molecular Medicine and Biochemistry.
- 3+ years of experience in biological research.
- Ability to self manage and self motivate.

Bioinformatics/Systems Biology PhD Student ***Job Code: BI-02***

To develop bioinformatics algorithms and tools.

- BSc/BCA/Diploma in Bioinformatics, Computer Science, Molecular Biology, Genetics or in other life sciences.
- Good skills in programming, algorithm development, database administration, statistics and modeling, with an interest in Biology.
- Experience in research and scientific publications are encouraged.

Data Analysis Scientist ***Job Code: DA-01***

To perform analysis and modeling/simulation of biological data using bioinformatics tools and methods of systems biology. To investigate molecular mechanisms of diseases and perform biomarker discovery.

- BSc/BCA/Diploma/PhD in Molecular Biology, Genetics, or Biochemistry.
- 3+ years of experience in biological research/service.
- Experience in applying Bioinformatics tools and databases to biological data.
- Confidence in using MS Office and databases.

Excellent English and communication skills are essential for all positions. All positions are located in our Wolfenbüttel, Germany office.

We accept complete applications only sent by eMail to:
hr@biobase-international.com

In your applications, please identify the job code and express your salary expectations.

For more information about the company please visit our homepage:
www.biobase-international.com

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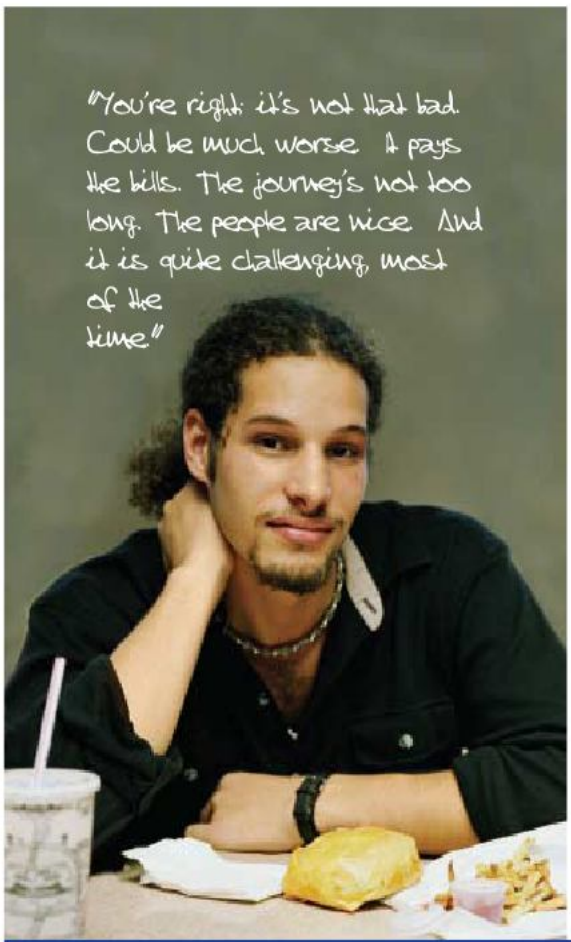

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Beaufort Marine Research Award

As part of the Irish Government's National Development Plan (NDP), the Beaufort Marine Research Award is grant aided by the Department of Communications, Energy and Natural Resources (DCENR) and the Department of Agriculture, Fisheries and Food (DAFF) under the Strategy for Science Technology and Innovation (SSTI) and the Sea Change Strategy.

Principal Investigator (PI) Ecosystem Approach to Fisheries Management (EAF)

Can you think and act outside the box?

The Marine Institute, together with its project partners at Queens University Belfast and University College Cork, is looking for a science leader and strategic thinker who will catalyse the application of the EAF to the waters around Ireland.

We are looking for a Principal Investigator (PI) who can harness and integrate the diverse research expertise required to develop a Centre of Excellence (CoE) on the Island of Ireland that focuses on the development and application of the EAF. Based at the Marine Institute, the PI will evolve the present scientific advice used by decision makers into pragmatic EAF based advice.

The fixed term contract will be for 7 years with a salary commencing at _ 92,000 increasing annually for the duration of the contract. A research grant of 30% of salary will also be available annually to the PI. Annual leave will amount to 30 working days. A detailed job description is available from Human Resources in the Marine Institute, Oranmore, Co. Galway, Ireland. This can also be downloaded from www.marine.ie Applications must reach the Marine Institute by 16:00 on Friday 14th March 2008.

The Marine Institute is an equal opportunities employer.

W123668R

Inflammation at Interfaces

Inflammation research represents one of the fastest growing academic disciplines with a large potential for translation into novel diagnostics and therapies. The two universities of Kiel and Lübeck, jointly with the Leibniz Center for Medicine and Biosciences (Borstel) and the Max Planck Institute for Evolutionary Biology (Plön), have therefore established the multi-disciplinary research network "Inflammation at Interfaces" for the study of mechanisms of chronic inflammatory diseases of barrier organs (e.g. lung, skin, intestine). Translation from genetic causalities into structural biology, pathophysiology and, finally, disease management, is the main scientific focus of the cluster. As part of this initiative, 12 new professorships (W1, W2 , W3) are being established, which we wish to fill with exceptional young researchers from the fields of medicine, natural sciences and nutrition sciences. The cluster of excellence will provide superb working conditions and infrastructures to its members with the goal to establish the leading scientific center for this topic in Europe.

Salary will be at grades W1, W2 or W3 as indicated. For positions designated as W1/W2, salary will be at grade W1 or at grade W2, depending on fulfilling legal and personal qualifications of the successful applicant. Each professorship also carries additional funding for scientific and technical support, personnel, and research materials.

The W3 and W2 professorships are initially appointed for 5 years with a performance interim review to decide about tenure (tenure track). The W1 Junior Professors will initially be appointed for 3 years ("Beamtenverhältnis auf Zeit"); dependent upon the performance of the Junior Professor after these three years, the position can be extended for up to 3 additional years. A tenure option (W2) can be offered for the W1 occupants according to further evaluation results.

The Christian-Albrechts-Universität zu Kiel (CAU) and the University of Lübeck (UzL) invite applications for the positions of

12 Professorships (W1, W2 and W3)

as part of the DFG-Cluster of Excellence "Inflammation at Interfaces"

1. W3 FOR MOLECULAR PREVENTION – FACULTY OF AGRICULTURAL AND NUTRITIONAL SCIENCES / MEDICAL FACULTY CAU:

To develop interventions based on molecular nutrition strategies, with the goal of the prevention of chronic inflammatory diseases. The validation of molecular epidemiology and genetic etiology, especially regulation and function of causative genetic variants, will be one important aspect. The group will be adequately equipped and will have access to patient facilities in the Comprehensive Center for Inflammation Medicine (CCIM) in addition to biobanking (popgen) and genotyping platforms. The applicant is also responsible for teaching the subject "Molecular Prevention". Applicants are expected to possess a 'Habilitation' or demonstrate equivalent scientific achievements in nutrition sciences or a related field.

2. W2 FOR EVOLUTIONARY GENOMICS OF THE INTESTINAL MICROFLORA – MEDICAL FACULTY CAU:

To study the interaction between evolutionary selection and the complex microflora in murine model systems, based on metagenomic approaches. The group is expected to work in the context of questions relevant to human diseases and will have access to the medical faculty and patient materials. Excellent candidates who have worked in related areas and who are willing to shift their research focus are encouraged to apply. The position will be hosted at the MPI Plön and the CAU.

3. W2 FOR CYTOKINE SIGNALLING – MEDICAL FACULTY CAU:

To study the modulation of the immune system by cytokines during inflammatory states with a strong focus on relevant animal models and gp130 signalling.

4. W2 FOR IMMUNOMODULATION – MEDICAL FACULTY UZL:

To study modulation of immunopathological processes involving the extracellular matrix (collagens) in the skin. Applicants must have broad knowledge in immunological and cell biological methods and experience with animal experiments. A pronounced interest in the transition of results from basic research into treatment concepts for patients is also expected. For this, the group will cooperate in particular with the Comprehensive Center for Inflammation Medicine (CCIM).

5. W1/W2 FOR GENETIC SUSCEPTIBILITY FOR GRANULOMATOUS DISEASE – MEDICAL FACULTY CAU:

To conduct systematic genetic and/or genomic studies identifying susceptibility mechanisms for sarcoidosis and other human granulomatous diseases. Excellent candidates who have worked in related areas and who are willing to shift their research focus are encouraged to apply. An intense collaboration between the university and the FZ Borstel supports the professorship.

6. W1/W2 FOR EPITHELIAL BARRIER FUNCTION – MEDICAL FACULTY CAU:

To conduct systematic genetic and/or genomic studies identifying genetic/genomic susceptibility mechanisms for inflammatory bowel diseases and other chronic inflammatory barrier disorders. Excellent candidates should have a track record in the field.

7. W1/W2 EPITHELIAL PROTEASE INHIBITORS – MEDICAL FACULTY CAU:

To conduct systematic studies on the role of epithelial protease inhibitors and proteases of epithelial and microbial origin in cutaneous inflammation and other inflammatory barrier diseases. Excellent candidates should have a track record in the field.

8. W1 FOR MOUSE MODEL SYSTEMS FOR DAMAGE AND INFECTION IN BARRIER ORGANS – MEDICAL FACULTY CAU:

To complement the existing models of aerosol tuberculosis (BSL3) and cutaneous leishmaniasis (BSL2). The Junior Professor is expected to have extensive experience in mouse models of infection and/or barrier challenge and, in collaboration with cluster researchers from the fields of dermatology, immunology, infection biology, and inflammation medicine, will newly establish and adapt attractive murine models to investigate the molecular pathogenesis and novel treatment modalities of chronic inflammatory diseases in skin, gut, lung or vasculature. An intense collaboration between the university and the FZ Borstel supports the professorship.

9. W1 FOR SYSTEMATIC FUNCTIONAL GENOMICS – MEDICAL FACULTY CAU:

To describe signalling events in innate immune receptor pathways with an emphasis on proteomic technologies and spatiotemporal analysis of post-translational modifications. Excellent candidates who have worked in related areas and who are willing to shift their research focus are encouraged to apply.

10. W1/W2 FOR GENETICS OF INFLAMMATORY SKIN DISEASE – MEDICAL FACULTY CAU/MEDICAL FACULTY UZL:

To conduct systematic genetic studies to discover susceptibility genes in immunobullous skin diseases. A large collection of patients with immunobullous diseases will be assembled for subsequent high density SNP association studies. Large patient samples with related chronic skin disorders have already been assembled and are available for association studies including availability of 500k SNP data. In addition, studies will include the backcrossing of mice susceptible to induction of autoimmune blistering with those resistant to disease.

11. W1 FOR STRUCTURAL BIOLOGY OF AUTOIMMUNITY – FACULTY FOR TECHNICS AND NATURAL SCIENCES UZL:

To analyze the structure of components of the skin matrix involved in autoimmunity using either crystallography or NMR spectroscopy. Candidates must have excellent knowledge in structural analysis of biological macromolecules.

12. W2 EPIDEMIOLOGY – MEDICAL FACULTY CAU:

To extend existing and establish new long-term prospective studies focussed on diseases of barrier organs (inflammation or other chronic disorders including cancer). Goals should include the study of genotype-phenotype relationships and the development of (non-genetic) biomarkers. Scientific direction of popgen, a population representative biobank (more than 40.000 individuals) with several large disease and control cohorts under study (www.popgen.de) will be part of the responsibilities. An academic certification or related fields in epidemiology would be advantageous.

■ Applicants must have the necessary formal qualifications as set out in §61 (W3 or W2 professorships) or in §64 (W1 professorships) of the University Law of Schleswig-Holstein and are expected to participate in teaching. The universities offer a family-friendly working environment and are proactive with respect to double-career families. The universities strongly encourage women with appropriate qualifications to apply for the positions. Women with equivalent qualifications, competence and expertise will be given preference. The state government and the universities support the employment of disabled persons. Persons with disabilities will, with appropriate qualifications and aptitudes, be employed preferentially. Applications, including a curriculum vitae, qualifying documentation (publications, evidence of external funding, teaching experience) and a short research plan should be submitted to the address below. Candidates willing to apply should request an application guideline at "inflammation@uv.uni-kiel.de" (subject: "EXC").

Closing date is 3 weeks after publication of this advertisement. Selected applicants will be invited to an extensive visit to the universities in Kiel and Lübeck and the partner institutes during the week of April 14–19, 2008. This will include a lecture and a formal interview.

Application for professorship 1 must be submitted to:
Prof. Dr. Joachim Krieter, Dean of the Faculty of Agricultural and Nutritional Sciences of Kiel University

Application for professorships 2–3, 5–10 and 12 must be submitted to:
Prof. Dr. Stefan Schreiber, Dean of the Medical Faculty of Kiel University

Application for professorship 4 must be submitted to:
Prof. Dr. Werner Solbach, Dean of the Medical Faculty of the University of Lübeck

Application for professorship 11 must be submitted to:
Prof. Dr. Enno Hartmann, Dean of the Faculty for Technics and Natural Sciences of the University of Lübeck

C|A|U
Christian-Albrechts-Universität zu Kiel

The postal address for all applications is:
Dr. Ina Plettner
Inflammation at Interfaces
Christian-Albrechts-Universität zu Kiel
Christian-Albrechts-Platz 4
24118 Kiel – Germany
Fax: ++49-(0)431-8801560
e-mail: iplettner@uv.uni-kiel.de

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www.inflammation-at-interfaces.de

Imperial College London

100 years of living science 100

Faculty of Medicine

Professorial Opportunities

Imperial College is ranked fifth in the top ten universities of the world, according to the 2007 Times Higher Education Supplement league tables.

To celebrate the 60th anniversary of the NHS and the creation of the UK's first Academic Health Science Centre, Imperial College London announces the creation of 30 new Professorial positions over three years.

These new appointments which are consistent with the strategic themes for Imperial College will work in the Faculty of Medicine and the new Imperial College Healthcare NHS Trust. These appointments underlines Imperial College's commitment to improving healthcare through research in both the basic and clinical sciences and demonstrates the advantages that the new relationship between universities and the NHS can bring to improving patient care.

The first tranche of professorial opportunities are in the following strategic themes:

- **Cardiovascular Science and Renal Medicine**
  - 2 Chairs in Cardiac Surgery and Developmental Dynamics
  - 1 Clinical Chair in Renal Medicine
- **Endocrine, Metabolism and Diabetes**
  - 1 Non Clinical Chair in Diabetes Research
- **Imaging/Rheumatology**
  - 1 Clinical Chair in Rheumatology and Musculoskeletal Imaging
- **Genetics and Genomic Medicine**
  - 1 Chair in Human Genetics
- **Translational Medicine**
  - 1 Chair in Haematology with an interest in haemopoietic stem cells
  - 1 Clinical Chair in Experimental Medicine
  - 1 Chair in Medical Statistics with an interest in clinical trial methodology
  - 1 Clinical Chair in Fetal Medicine

The successful applicants will have an international standing, outstanding publication record, an established record of raising research funding, experience in managing and delivering research projects and/or programmes, and substantial experience of supervising and inspiring junior researchers.

For an informal discussion on any these positions please contact the following Heads of Division in the first instance:

Caradiovascular Science – Professor Anthony Newman Taylor, a.newmant@imperial.ac.uk

Renal Medicine, Diabetes Research and Genetics and Genomic Medicine - Professor Jonathan Weber, j.weber@imperial.ac.uk

Fetal Medicine – Professor Mervyn Maze, m.maze@imperial.ac.uk

Rheumatology and Musculoskeletal Imaging – Professor Marc Feldmann, m.feldmann@imperial.ac.uk

Translational Medicine – Professor Martin Wilkins, m.wilkins@imperial.ac.uk

Further particulars for each position and an application form (which must be completed) can be obtained from the following link http://www.imperial.ac.uk/employment/academic/index.htm

Alternatively, please contact Maria Monteiro, Senior Appointments Coordinator (Professors and Readers), Human Resources Division, Level 3 Faculty Building, Imperial College London, London SW7 2AZ. email m.monteiro@imperial.ac.uk quoting reference number **RB02-08.**

Closing date: 28 February 2008.

Valuing diversity and committed to equality of opportunity

U123894RM

ncbs

national centre for biological sciences
tata institute of fundamental research

5 year International Investigator position in collaboration with Merck & Co.

Recognizing the quality of intellectual and other resources available at NCBS to address major questions in modern biology, Merck & Co., Inc. (Whitehouse Station, NJ, USA; operating in India as MSD Pharmaceuticals, Ltd.) has collaborated with NCBS to institute the NCBS-Merck & Co., International Investigator position. This investigator will be a new recruit at NCBS, on a 5 - year non-renewable appointment. All the necessary infrastructure and logistical support normally given to a new investigator will be provided by NCBS. The position and salary, also provided by NCBS, will be at the level of a starting faculty/young investigator appointment at NCBS depending upon the experience and qualifications of the selected candidate. The NCBS-Merck & Co., International Investigator will additionally receive a US $40,000 prize award each year by Merck & Co., for a maximum period of 5 years, based on annual review. This appointment is not linked to a continuing position at NCBS. Should the selected researcher choose, at any stage, to apply for a tenured position at NCBS, this will be independently evaluated.

The Investigator's research and scientific directions are expected to bring a new and interactive area of biomedical research to NCBS. Specifically, we are looking for candidates of the highest-quality, with excellent publications and track-record in basic biomedical sciences and, importantly, with interests in - and a commitment to - the development and management of platform-technologies, which are driving the frontiers of modern biology. NCBS will provide all the support required for the establishment and running of these platforms.

A search will be carried out till a suitable candidate is found. Applications are invited for this position from researchers of any nationality. Nominations of suitable candidates, who can be contacted, are also welcome.

Interested applicants should submit a CV, brief research proposal and names of referees to:

Professor Jayant Udgaonkar, Dean, NCBS
(dean@ncbs.res.in)

Details on the research being carried out at NCBS are available on our website:
www.ncbs.res.in

National Centre of Biological Sciences
Tata Institute of Fundamental Research
GKVK, Bellary Road
Bangalore – 5600 065 INDIA
Ph. +91 80 23666001 / 02 / 18 / 19
Fax +91 80 23636662

RW123335R

The Paterson Institute is a leading cancer centre of excellence core-funded by Cancer Research UK and is an Institute of The University of Manchester.

Paterson
Institute for Cancer Research

Postdoctoral Scientists

Salary in the range of £25,500 - £38,000 dependent upon qualifications and experience

IMMUNOLOGY GROUP - Reference number: PI/08/06

A three year position is available within the Immunology Group. The group's basic studies of the 5T4 oncotrophoblast antigen have shown the molecules to be intimately associated with epithelial-mesenchymal transition, a process which is important in development and contributes to metastasis in cancer (J Cell Sci. 116: 4533-42, 2003; Exp Cell Res 312:1713-26, 2006; Mol. Biol. Cell, 18: 2838-51, 2007; Cancer Res. 67:11254-62, 2007). This position will focus on the down-stream consequences of the functional interactions of 5T4 molecules at the cell membrane and with the cytoskeleton utilising embryonic stem cell models to investigate the key signalling pathways.

A background in the biochemistry of motility or adhesion would be an advantage.

Informal enquiries should be directed to Professor Peter Stern on 0161 446 3127, email: pstern@picr.man.ac.uk

STROMAL-TUMOUR INTERACTION GROUP - Reference number: PI/08/08

A three year position is available to study tumour microenvironment. The major aim of the laboratory is to elucidate paracrine signals by which tumour microenvironment confers metastatic and self-renewing propensity into nearby carcinoma cells. Stroma-targeted therapeutic approach will be also developed in transgenic mouse tumour models.

References:

1. Orimo A. and Weinberg RA. (2006) Stromal fibroblasts in cancer: a novel tumor- promoting cell type, Cell Cycle, 5, 1602-1606.
2. Orimo A., et al., (2005) Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion, Cell, 121, 335-348.

You will have a PhD in Cell Biology or related field and three years' experience in Cell Biology, Molecular Biology or Biochemistry.

For information on the Stromal-Tumour Interaction Group, please visit www.paterson.man.ac.uk/stromal/

Informal enquiries should be directed to Dr Akira Orimo: aorimo@picr.man.ac.uk

CLINICAL AND EXPERIMENTAL PHARMACOLOGY GROUP - Preclinical Pharmacologist in Childhood Cancer - Reference number: PI/08/09

A three year position is available from 1st April 2008 for a motivated and enthusiastic Postdoctoral Scientist to join a team of researchers in the Clinical and Experimental Research Group within newly refurbished, purpose-built translational research laboratories led by Professor Caroline Dive. You will join the paediatric cancer focus group led by Dr Guy Makin evaluating novel anticancer drugs and link to the clinical teams who undertake biomarker enhanced early clinical trials of mechanism based anticancer drugs in childhood cancer.

Ideally you will have two years' postdoctoral experience within a biology, biochemistry or pharmacology laboratory. You will have expertise in the biochemical analysis of cellular signalling cascades. Specifically, you will study signalling transduction pathways and the impact of tumour micro-environmental factors on drug-efficacy in human paediatric cancer cells. You will work in a project team that studies novel anticancer drugs targeted to apoptosis regulatory proteins.

Informal enquiries should be directed to Professor Caroline Dive on 0161 446 3036, email: cdive@picr.man.ac.uk or Dr Guy Makin on 0161 922 2227 email: guy.makin@manchester.ac.uk

For further information and to apply for these positions, please visit our website. For applicants who are unable to download this information from our website, please contact Laura Humes, HR Assistant on 0161 446 3124, email: lhumes@picr.man.ac.uk to have this information sent by post.

Closing date for submission of applications is Thursday 21 February 2008

www.paterson.man.ac.uk

MANCHESTER 1824
The University of Manchester

CANCER RESEARCH UK

U123902R

Universität Hamburg

Helmholtz Department of Structural Biology at DESY

The Helmholtz Association (HGF) is Germany's largest research agency, encompassing 15 research centers and an annual budget of around 2.3 billion Euro.

DESY, the German Electron Synchrotron in Hamburg, is a member of the Helmholtz-Association. In 2009 DESY will re-dedicate the storage-ring PETRA III, providing the most brilliant X-ray source worldwide. Three of four X-ray beamlines, assigned to biological research, will be available for protein crystallography. The X-ray free electron laser X-FEL, scheduled to begin operation in 2013, will additionally allow for unprecedented real-time research into biological processes at the atomic scale.

The Helmholtz Association and the University of Hamburg invite applications for:

W3-PROFESSOR FOR STRUCTURAL BIOLOGY AND HEAD OF THE HELMHOLTZ DEPARTMENT OF STRUCTURAL BIOLOGY (Code/Kennziffer 1968/W3)

The department head will play a critical role in setting up a new Helmholtz department on the DESY campus and is expected to develop and employ novel approaches to address fundamental questions relevant to the health research of the Helmholtz Association, including e. g. infection biology, cancer, metabolic disorders and/or neurodegenerative diseases. The department head will also be responsible for managing a world-class PX beam-line at PETRA III jointly with the Max-Planck-Society, providing support to research groups from the Helmholtz Association and beyond.

Basic requirements:
According to § 15 Hamburgisches Hochschulgesetz.

We seek highly motivated candidates with an outstanding scientific track record, international reputation in protein crystallography, experience in managing a research group, and a demonstrated ability to recruit third party funds. The position is tenured and will be a joint appointment (professorship at W3-level) with the University of Hamburg. Generous funds (personnel, investments, running costs) to establish an independent, state-of-the-art structural biology laboratory on the DESY campus will be provided.

JUNIOR PROFESSORSHIP AND HEAD OF A JUNIOR RESEARCH GROUP IN STRUCTURAL BIOLOGY (Code/Kennziffer JP97)

The head of the junior research group is expected to develop innovative and competitive research highly relevant to human health.

The position will be a joint appointment (junior professorship at W1-level) with the University of Hamburg. Junior professors will initially be appointed for 3 years and can be extended by up to 3 additional years on the basis of a positive evaluation of academic performance.

Funding will be provided for the group leader, a post-doctoral scientist, a PhD student and a technician.

Basic requirements:
According to § 18 Hamburgisches Hochschulgesetz.

The candidate for the group leader should have an excellent scientific track record and postdoctoral experience in protein crystallography. The research group will be accommodated within the Helmholtz Department of Structural Biology at DESY.

Teaching obligation for both positions: 2 hours per week. Experience in teaching is required. The University of Hamburg will run a graduate program "Molecular Life Sciences".

The University of Hamburg and the Helmholtz Association are equal opportunity employers and welcome applications from all qualified individuals. They aim at increasing the representation of women among their staff and therefore explicitly encourage applications from female scientists. Disabled persons are given priority over applicants of equal suitability, qualification and degree of specialized knowledge.

For full consideration, applications (including a CV, a complete list of grants and publications, and a summary of current and future research interests and objectives) should be send by **6th March 2008** to the President of the University of Hamburg, Ref. 631.6, Moorweidenstraße 18, 20148 Hamburg, Germany.

For further information please contact Prof. Dr. Dirk Heinz, Division of Structural Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany (dirk.heinz@helmholtz-hzi.de).

W123434R

TOR ZUR WELT DER WISSENSCHAFT

Australian Government Bureau of Meteorology

The Centre for Australian Weather and Climate Research
A partnership between CSIRO and the Bureau of Meteorology

CSIRO

Director

- Package to approx $220K
- Location: Melbourne, Australia

The Centre for Australian Weather and Climate Research is a partnership between Australian's leading atmospheric and oceanographic research agencies; the Bureau of Meteorology (BoM) and CSIRO.

Given the importance of integrating the science, the partners' vision is to create a joint Centre for earth system science recognised for its innovation and scientific excellence nationally and as a world leader in its field. The Centre is being supported for an initial 5 year period.

We are seeking a Director for the Centre, responsible for providing leadership in delivery outcomes against the Centre's long term Strategic Science Plan and Annual Plan through the collaborative partnership of CSIRO and the BoM (the Participants) by leveraging and enhancing earth systems science and atmospheric science capability of both organisations for the benefit of Australians, Australian Industry and society.

The key areas of impact for this position are:

- Delivery of research outcomes of national significance;
- Strategic management of science capability; and
- Leadership in Australian earth systems science.

This senior leadership appointment will be offered for a 4 year period, renewable depending on the success of the outcomes of the Centre. The successful candidate will be employed by CSIRO or the Bureau of Meteorology by negotiation.

For further details about the position please contact Dr Chris Mitchell on (+61) 03 9239 4673 or visit our website at **www.cawcr.gov.au** for information about the Centre.

For selection documentation and details on how to apply visit **www.csiro.au/careers** or call **1300 301 509**.

JP123857R

hmaC078112

UNIVERSITY OF LIVERPOOL

School of Clinical Sciences
Division of Metabolic and Cellular Medicine

Postdoctoral Researcher

£28,289 - £42,791 pa

You will join the well-funded Cell Pathologyphysiology Research Group, studying reactive oxygen species and the age-related loss of skeletal muscle mass and function, and also undertaking basic mechanistic studies into skeletal muscle physiology and pathologies. You should have a PhD in a relevant area of biology together with postdoctoral research experience. Experience of confocal fluorescence microscopy and cell transfection techniques would be advantageous. The post is available for 3 years and is funded by the Medical Research Council.

Job Ref: R-567202/N **Closing Date: 29 February 2008**

School of Biological Sciences

You will join a 3-year BBSRC-funded project **"Sequencing the trancriptome of Kalanchoe fedtschenkoi: a model for Crassulacean acid metabolism (CAM), embryogenic plantlet formation and the Saxifragales"**. This is a ground-breaking project that will employ the latest ultra high-throughput pyrosequencing technology (454 GS FLX) to achieve deep sequencing of the transcriptome of *Kalanchoe fedtschenkoi*.

Postdoctoral Researcher

£28,289 - £29,139 pa

You will generate samples for 454 sequencing, perform digital northern analysis, generate transgenic lines to manipulate key CAM genes, and undertake detailed phenotypic analysis.

You should have (or be about to obtain) a PhD with experience in plant molecular biology, biochemistry and/or physiology with preference being given to individuals with experience of analysis of large genomics data sets. Ideally, you will also have experience of generating plant binary constructs, and working with non-model plant species.

Job Ref: R-567182/N **Closing Date: 22 February 2008**

Research Technician Grade 5

£20,458 - £22,332 pa

You will be responsible for the growth and maintenance of *K. fedtschenkoi*, isolation of RNA and DNA samples, generating transgenics and phenotype analysis.

You should be qualified to BTEC Higher level (or equivalent) with experience in plant molecular biology, biochemistry and/or physiology with preference being given to individuals with experience of tissue culture based plant transformation. Ideally, you will also have experience of PCR, RT-PCR, and enzyme and metabolite assays.

Job Ref: S-567180/N **Closing Date: 22 February 2008**

U123899R

For full details, or to request an application pack, visit
www.liv.ac.uk/working/job_vacancies/
Tel 0151 794 2210 (24 hr answerphone)
Please quote Job Ref in all enquiries.
COMMITTED TO DIVERSITY AND EQUALITY OF OPPORTUNITY

UNIVERSITY OF SURREY

Chair in Nutrition

Nutritional Sciences Division
Faculty of Health and Medical Sciences

You will have an established, internationally-recognised research profile in understanding the metabolic demands for nutrients in health and disease and the optimisation of nutrient provision in the form of appropriate food components. The appointee will also have a substantial track record both in attracting research funding and in publication in high impact journals.

Within the Faculty, the Nutritional Sciences Division is one of the UKs foremost academic nutrition centres, with a multidisciplinary group of 18 academic staff. Currently 5* RAE rated, our research programme integrates its scientific and clinical disciplines into cross cutting multi-disciplinary Faculty themes, focussing on human health, disease and treatment. Over the last 5 years, £8 million has been invested in refurbishments and equipment, in Functional Genomics, Proteomics and Metabonomics, Neuroscience, Experimental Biology, Materials Research and Clinical Science.

You will be expected to develop a high profile research programme in Nutrition which complements the current research activities of the Division and to contribute to both undergraduate and postgraduate teaching. A research interest appropriate to Nutritional Medicine would be particularly welcome.

The appointee will report in the first instance to the Head of the Division of Nutritional Sciences (Professor Linda Morgan) and ultimately to the Dean of Health and Medical Sciences (Professor John Hay).

For informal discussions, please contact The Head of Division, Professor Linda Morgan (l.morgan@surrey.ac.uk) or the Professor of Nutritional Medicine, Professor Margaret Rayman (m.rayman@surrey.ac.uk).

Our benefits package includes generous annual leave (25 days plus a further seven closure days plus bank holidays) relocation provision, final salary pension scheme, childcare assistance and leisure facilities.

Please apply online, or contact Christina Kirby on 01483 684596 or email: FHMS_HumanResources@surrey.ac.uk quoting ref: 6446. On making a formal application for this post, applicants must include a description of their current research activities and an outline of the research programme to be pursued on appointment (two pages maximum).

Closing date: 29 February 2008.

We acknowledge, understand and embrace cultural diversity

www.surrey.ac.uk/vacancies

U123863R

estools
Advances with Human Embryonic Stem Cells

Call for additional partner to enhance bioinformatics data storage and analysis in ESTOOLS, a consortium for an EU 6th Framework Programme Integrated Project.

Project title: Platforms for biomedical discovery with human ES cells.

Activity area: ESTOOLS aims to advance the fundamental knowledge that will underpin biomedical application of human embryonic stem cells by developing tools and procedures to facilitate (1) unlimited expansion of hESCs (2) creation of techniques for the efficient, regulatable expression and ablation of hESC gene function (3) directed differentiation of hESCs into mature neural cell types exhibiting full phenotypic fidelity.

Call announcement: We seek a new partner, established in an EU Member State or Associated State, with expertise in these areas: databases of gene expression data; statistical analyses of gene expressions or array data; data integration; conducting training in bioinformatics techniques.

Budget: EC funding available up to €300,000.

Expected duration of participation: to July 2010.

Project Coordinator: Professor Peter W. Andrews.

Additional information about ESTOOLS and the Call: visit www.estools.eu or email call@estools.eu

Proposal submission address: ESTOOLS Call, Mr Andrew J.T. Smith (Project Manager), BMS / c227 Alfred Denny Building, University of Sheffield, Western Bank, Sheffield S10 2TN, UK.

Close of Call: 12 March 2008 at 17:00 GMT.

U123851R

Change your environment. Find jobs where you'll make a difference

naturejobs

csbe centre for systems biology at edinburgh

THE UNIVERSITY of York

bbsrc biotechnology and biological sciences research council

THE UNIVERSITY OF EDINBURGH

UNIVERSITY OF LIVERPOOL

EPSRC Engineering and Physical Sciences Research Council

THE UNIVERSITY OF WARWICK

Vacancies In Integrative Systems Biology

The £6M ROBuST project is recruiting a high-calibre interdisciplinary research team to model the effects of temperature on a defined signalling network in Arabidopsis thaliana. This large integrative project brings together biologists working on molecular signalling and climate change, Mathematicians and Informaticians. Excellent facilities are available for the modelling of dynamic biological processes and using the techniques of computational biology, for molecular genetics, biochemistry and high throughput data production. The project management team will comprise: Halliday/Millar/Goryanin/Williams (Edinburgh), Rand/Finkenstadt (Warwick), Penfield/Graham (York), Hall/White (Liverpool).

Positions available (closing date 14 February 2008)

1. One Lead Experimental Postdoctoral Research Associate (Edinburgh)
- Expertise: plant molecular genetics, protein production, interdisciplinary work, experienced supervisor.

2. Two Theoretical Postdoctoral Research Associates (Edinburgh, Warwick)
- Expertise: mathematical biology and/or computational systems biology.

3. Two Experimental Postdoctoral Research Associates (York, Liverpool)
- Expertise: plant molecular genetics, microscopy (Y), interdisciplinary work

4. Four Lab-based Technicians (Edinburgh, York, Liverpool)
- Expertise: Arabidopsis, plant genetics, molecular biology techniques, spreadsheets, must be highly organised

5. One Administrator (Edinburgh)
- Expertise: knowledge of University, grant and finance administration

For more information :

http://csbe.bio.ed.ac.uk/ROBuST%20project/ROBuST_project.htm

All posts are available through www.jobs.ac.uk

Contacts at our four sites

Edinburgh: Dr Karen Halliday (e-mail: karen.halliday@ed.ac.uk)
Warwick: Professor David Rand (e-mail: dar@maths.warwick.ac.uk)
York: Dr Steve Penfield (e-mail: sdp5@york.ac.uk)
Liverpool: Dr Anthony Hall (e-mail: anthony.hall@liverpool.ac.uk)

U123923R

UCD DUBLIN

A University to match your ambitions

UCD Conway Institute of Biomolecular and Biomedical Research

Director of Biological Imaging

Ref: 003271

Fixed-term 5-year post

The UCD Conway Institute for Biomolecular and Biomedical Research is the flagship biomedical research facility for University College Dublin. It is housed in a modern purpose built building located on the Belfield Campus. The building houses approximately 450 research scientists including approximately 200 PhD students in the Life Sciences. Research in the Conway Institute is supported by access to state-of-the-art core technology infrastructure including proteomics, genomics, imaging (single cell and model organisms) and bioinformatics.

We are looking to appoint a Director of Biological Imaging to work with the expert core technology staff and academic staff in the Conway Institute to optimise the use of the investment in the imaging infrastructure including cell based imaging systems and in vivo imaging. The Director will also be expected to develop strong links with the industry providers of equipment to ensure that the facility at the Conway remains at the cutting edge.

Salary will commensurate with qualifications and experience.

W123756R

Ireland's education capital.

University College Dublin
An Coláiste Ollscoile,
Baile Átha Cliath

Further particulars and application procedures for these posts are available at www.ucd.ie/vacancies or from UCD HR.
UCD HR, University College Dublin, Belfield, Dublin 4, Ireland.
Tel: 00-353-1-716 4900; Fax: 00-353-1-716 4949

www.ucd.ie/vacancies

UCD is an equal opportunities employer.

University of Reading

www.reading.ac.uk/jobs

RCUK Academic Fellow

SBS/SCFP (Pharmacy)

This appointment is full-time, permanent and starts 1 April 2008 or as soon as possible
Grade 6/7 £25,134 to £41,545 per annum

We invite applications for an RCUK Fellowship in emerging technologies for systems biology to complement earlier awarded fellowships in integrated systems biology.

We seek an innovative researcher with expertise in the application of systems biology approaches specifically in the areas of cardiovascular and metabolic research. This may include (but is not restricted to) the study of vascular and cardiac biology, haemostasis and thrombosis, obesity, metabolic syndrome, and type II diabetes.

You will have:

- a PhD in a Life or Physical Sciences subject, and postdoctoral experience of systems biology studies
- an aptitude for multi-disciplinary collaborative research, at the interface between biological/physical techniques, mathematics and computational modelling
- experience of study design, and the generation, management, and analysis of large data sets (e.g. (pharmaco)genetic profiling, gene transcriptional analysis, proteomics)
- experience with bioinformatic data analysis resources
- experience in developing and independently undertaking research projects
- the ambition to excel in academic research

Candidates with expertise in both post-genomic technologies and mathematical modelling of biological/pharmaceutical data are strongly encouraged to apply.

Informal enquiries: contact the Head of Biomolecular Sciences, Professor Jon Gibbins on +44 (0)118 378 7082 or email j.m.gibbins@reading.ac.uk alternatively, contact the Head of Pharmacy, Professor Gavin Brooks on +44 (0)118 378 4637 or email g.brooks@reading.ac.uk

Closing date: 28 February 2008

Further information and application forms are available at www.reading.ac.uk/jobs, or from:

Human Resources, University of Reading, Whiteknights, PO Box 217, Reading RG6 6AH,
Telephone 0118 378 6771 (voicemail)

Please quote reference number RS08006

We value a diverse workforce and welcome applications from all sections of the community

The Queen's Anniversary Prizes 2005

U123896R

University of Oxford

Department of Cardiovascular Medicine & Wellcome Trust Centre for Human Genetics

Postdoctoral Research Fellows (2 Posts)

£26,666 - £32,796 p.a.

"Downstream mechanisms in hypertrophic cardiomyopathy"

We are looking for two experienced and highly-motivated postdoctoral research fellows who would like to develop their scientific skills in a multidisciplinary research group with a focus on mechanisms of human disease. Our group has been investigating an inherited heart muscle disorder (hypertrophic cardiomyopathy) to understand disease pathways and explore potential new therapies, that we hope will also be valuable in more common forms of cardiac hypertrophy and heart failure (see Ashrafian et al. Trends Genet. 2003;19:263-8; Robinson et al. Circ Res. 2007;101:1266-73).

Our work is funded by a recently renewed British Heart Foundation programme grant which aims to investigate the importance of primary and secondary changes in energetics, angiogenesis and calcium handling in cardiomyopathy.

Post 1: will have responsibility for the creation and analysis of murine models (including TET-inducible transgenic lines). You will hold a PhD in a relevant discipline, with substantial molecular biology experience. You will be responsible for the molecular and histological analysis of the model and for data analysis (but not acquisition) of phenotyping studies including MR imaging and spectroscopy. Experience of creating genetically manipulated models would be an advantage.

Post 2: will have responsibility for molecular and metabolic manipulations to investigate the interactions of anaplerotic metabolism, HIF-signalling and angiogenesis. You will hold a PhD in a relevant discipline, with substantial experience of cell and molecular biology, ideally including tissue culture analyses (including molecular biological protocols, e.g siRNA, real time PCR); you will also be involved in ex vivo analyses of genetically manipulated murine models. An interest in metabolism would be an advantage.

For both posts, you would need to be well-organised and able to interact with postdoctoral scientists and graduate students within our group, and with collaborating groups. There are excellent opportunities for personal and career development within the group and in the Department. Both posts are for 3 years with the possibility of extension.

Further particulars, which detail the application procedure, should be obtained from http://www.admin.ox.ac.uk/fp/ Informal enquiries may be made to Prof. Hugh Watkins (e-mail: hugh.watkins@cardiov.ox.ac.uk). The closing date for applications is 22 February 2008.

The University is an Equal Opportunity Employer

U123924RM

www.ox.ac.uk/jobs

National University of Singapore Graduate School for Integrative Sciences and Engineering

NUS National University of Singapore

Appointment of Executive Director

The NUS Graduate School for Integrative Sciences and Engineering (NGS) (http://www.nus.edu.sg/ngs) is a premier graduate school of the National University of Singapore (NUS), a leading university in Asia and globally. NGS offers opportunities to top quality students for interdisciplinary PhD research with carefully-selected supervisors. Students also undertake cross-disciplinary coursework tailored to students' individual needs and interests. NGS now seeks an **Executive Director,** responsible to the Office of the President, for the operations of NGS. The Director should be a distinguished scientist, engineer, or clinician-scientist with a strong record of interdisciplinary research in science, engineering, computer science, or biomedical research. He/she should also have strong interest in, and experience of, developing excellent graduate programmes. It is expected that the Director will hold simultaneous senior academic positions in one or more of the relevant Faculties/Schools at NUS (Engineering, Science, Medicine, Computing).

Applications with full CV and the names of at least three referees should be sent to the Chair of the Search Committee at the following address:
Professor W. R. Schowalter
c/o Ms Wu Yilian
Office of the Deputy President (Research and Technology),
National University of Singapore
University Hall
Lee Kong Chian Wing, UHL #05-02H
21 Lower Kent Ridge Road
Singapore 119077

Informal/Confidential enquiries can be made to Professor Schowalter (uprwrs@nus.edu.sg) or to Professor Barry Halliwell (bchbh@nus.edu.sg), the current Executive Director of NGS and Deputy President (Research and Technology) at NUS.

JP123395R

wellcome trust sanger institute

U123753RL

Wellcome Trust Sanger Institute
Pathogen Sequencing Unit (PSU)
Senior Computer Programmer

Position for a senior programmer to join the software team which supports the genome resource developed in the PSU to provide plug-ins and viewing and transferring annotation between organisms and improved graphics for the site. A degree in biology/computer science and ability to apply object oriented programming in a UNIX/LINUX environment essential.

Contact
Human Resources
E-mail: recruit@sanger.ac.uk
Web: www.sanger.ac.uk

MRC Medical Research Council

MRC Laboratory of Molecular Biology, Cambridge

Programme Leader Track

£37,000 - £47,000 pa

Applications are invited for a Programme Leader Track position to lead a new research group in the Cell Biology Division of the Laboratory of Molecular Biology. The LMB has an exceptional record of ground-breaking research, a broad remit, and excellent resources.

Although the primary criteria for selection are scientific excellence and potential for major impact, we are particularly interested in candidates solving fundamental and mechanistic questions of cell biology or development with biochemical or biophysical techniques. Another important factor is the potential to synergise with, or complement, existing members of the division, and we ask candidates to refer to this in their covering letter.

You should have completed a period of postdoctoral training, with an excellent track record, showing outstanding potential for independent research. You will lead a small team and substantial funding will be available.

Appointment will be made at either Programme Leader or Programme Leader Track depending on experience and achievements. Further information about this position is available from Matthew Freeman, email: mf1@mrc-lmb.cam.ac.uk or Mariann Bienz, email: mb2@mrc-lmb.cam.ac.uk

You will be supported by a flexible pay award policy, 6 weeks annual leave and public holidays, optional MRC final salary pension scheme and excellent onsite sports and social facilities.

This position is subject to pre-employment screening.

Applications for this post must be made online at http://jobs.mrc.ac.uk inputting reference number LMB08/030. If you do not have access to the internet or experience technical difficulties please contact 01793 301280.

Applications should include a covering letter and full CV, an outline of current research interests (1 page) and a proposal for future research (up to 2 pages), along with the names and addresses of three professional referees who have agreed to be contacted prior to interview.

Closing date: 7 March 2008.

For further information about the MRC visit www.mrc.ac.uk

The MRC is an Equal Opportunities Employer

'Leading science for better health'

U123911R

giraffe

Experienced Pharmacologist

Attractive Package
Thames Valley

A world leader in innovative drug discovery and development, our client seeks an accomplished scientist/senior scientist to join their world-class team at a rapidly expanding UK research facility.

You will design, undertake and report in-house pharmacology studies, applying your talents to a range of therapeutic targets within drug discovery. You will also contribute to strategic planning, and liaise with the company's international parent group, the academic world, CROs and regulatory authorities.

An experienced post-doctoral pharmacologist and a natural problem solver, you will have extensive first hand knowledge of *in vivo* models of oncology and/or inflammation/immunology, holding (or having recently held) a Home Office licence. You are proactive and an enthusiastic team player, able to combine the demands of a laboratory-based workload with significant project and academic commitments.

Please send your CV to maria@giraffeads.com.

We would appreciate all applications by our closing date of Friday 29th February 2008.

www.giraffeads.com

U123917R

Translational Medicine Research Collaboration (TMRC)

DIRECTOR OF BIOMARKER DISCOVERY

We are seeking to appoint an outstanding individual to this newly created post within the TMRC. The TMRC is a collaborative network linking Centres of Excellence in clinical and basic science at the Universities of Aberdeen, Dundee, Edinburgh and Glasgow and their respective NHS boards with Wyeth's R&D division to facilitate the development of novel medicines.

You will be expected to establish a translational research program of international standing in one or more of the following areas: cardiovascular, oncology, inflammation, musculoskeletal biology or neuroscience. The candidate would be expected to utilize such expertise to further develop effective interdisciplinary collaborative research within the TMRC, across Scotland. They will also be expected to actively contribute to the development of a translational medicine curriculum.

The successful candidate will also be expected to actively contribute to the development of novel biomarkers in the TMRC Laboratory located at Ninewells Hospital and Medical School, University of Dundee. Experience in the analytical validation and clinical development of in vitro diagnostics assays is highly desirable.

Candidates must have completed a first-rate PhD or MD degree in medicine, veterinary medicine, biology or related field. The appointee should be internationally recognised for research activities in their field with a strong translational orientation and should have a proven ability to attract grant support and publish in high quality journals. Suitably qualified individuals will be offered a Professorship in Translational Medicine.

This post will be based temporarily at the JBC at the University of Dundee, but will relocate to the new facility at Ninewells Hospital that will be completed by end of 2008. The appointment will be based on a three year, renewable contract.

For more information about the position and application instructions contact Professor Andrew Morris, Head of Planning and Development, College of Medicine, Dentistry and Nursing; a.d.morris@dundee.ac.uk; or Professor Mike Ferguson, Dean of Research, College of Life Sciences, University of Dundee; m.a.j.ferguson@dundee.ac.uk.

Interviews are likely to be held at the end of February 2008.

Applications in the form of a CV and covering letter, including the names and addresses of 3 referees, should be sent to HR-LifeSciences@dundee.ac.uk quoting LS/2086/N. Alternatively, please send 2 copies of your CV and covering letter to Human Resources, College of Life Sciences, MSI/WTB/JBC Complex, University of Dundee, DD1 5EH. Closing Date: 12 February 2008.

As part of the recruitment process, the University requires that a Disclosure Scotland check is undertaken for this position.

The University of Dundee is committed to equal opportunities and welcomes applications from all sections of the community.

U123918R

UNIVERSITY OF DUNDEE

www.dundee.ac.uk/jobs

School of Biological Sciences

The School of Biological Sciences (http://www.rhul.ac.uk/biological-sciences) constitutes 3 highly-integrated and dynamic research centres: Biomedical Sciences, Plant Molecular Sciences; and Ecology, Evolution & Behaviour. In addition, staff are associated with Faculty-based inter-departmental research centres in Computational Biology, Neuroscience and Nanobiotechnology. The School holds grade 5 research assessment (2001 RAE) status, and a maximum grade 24 teaching quality assessment (QAA) rating. Research and teaching activities are underpinned by technologies spanning functional genomics, metabolomics, molecular genetics, microbiology, gene therapeutics, computational & systems biology, advanced cell biology, model organism analysis, population studies, ecology, evolutionary biology, mathematical biology and advanced microscopy facilities.

Applications are invited for five academic posts in the School.

Lecturers in Biomedical Sciences (2 posts)

Ref: KB/4926

Applications in the fields of neuroscience, cell signalling, molecular microbiology/parasitology, infection and immunity or epidemiology are especially welcome.

Lecturer in Chemical Biology

Ref: KB/4927

Candidates should have a strong background in chemistry to contribute to bioscience chemistry teaching and research experience especially in the fields of medicinal chemistry, nutraceuticals, natural product chemistry or cell signalling.

Professor/Reader in Neuroscience

Ref: KB/381

Applicants should have expertise in molecular and cellular neuroscience or neurophysiology.

Senior Lecturer/Reader in Biological Sciences

Ref: KB/4928

Applicants with particular interest in the areas of behaviour/behaviour ecology, modelling or ecological genomics are especially welcome.

Informal enquiries are welcome to the Head of School, Professor Peter Bramley (tel. +44 (0)1784 443555; email: p.bramley@rhul.ac.uk)
Salaries in the range of: Lecturer: £36,927 - £47,531 p.a. Senior Lecturer: £44,925 - £54,769 p.a. Professorial salaries are negotiable starting at £53,229 p.a. All salaries are inclusive of London Allowance.
All posts are tenable from 1 September 2008 unless otherwise stated in the further details.

The closing date for all posts is 27 February 2008. Further details and a copy of the application form are available at www.rhul.ac.uk/Personnel/JobVacancies.htm

We positively welcome applications from all sections of the community.

Royal Holloway
University of London

U123915RM

MRC LABORATORY FOR MOLECULAR CELL BIOLOGY

MRC Medical Research Council

CAREER DEVELOPMENT FELLOW

The MRC Laboratory for Molecular Cell Biology is an internationally renowned molecular and cell biology institute situated at University College London **(http://www.ucl.ac.uk/LMCB/)**

We are seeking a highly motivated and creative scientist to join the team led by Dr Antonella Riccio, to study novel mechanisms of gene expression in neurons.

For more information about the research topics please visit:
http://www.ucl.ac.uk/lmcb/research-groups/riccio.htm
http://www.ucl.ac.uk/biology/academic-staff/riccio/riccio.html

Candidates should possess an MD and/or a PhD degree in a relevant biomedical discipline. Experience in molecular biology and mouse genetics is preferred, a background in transcriptional mechanisms would be an advantage.

Available in April 2008, the post is offered on a fixed-term contract of up to three years in the first instance. The starting salary will be on Band 4 from £28,877 to £34,829 inclusive, depending on previous experience.

Benefits include 30 days annual leave, contributory final salary pension scheme and an interest free season ticket loan.

Applications for this role must now be made online at **http://jobs.mrc.ac.uk** If you do not have internet access or experience technical difficulties please call 01793 301049 quoting reference CBU07/041.

Closing date for applications: 28 February 2008

'Leading Science for Better Health'

The Medical Research Council is an Equal Opportunities Employer and operates a strict no smoking policy.

U123348R

Friedrich Miescher Institute for Biomedical Research

Position in Bioinformatics / Computational Biology

Applications are invited for a position in the Bioinformatics department of the Friedrich Miescher Institute for Biomedical Research (FMI). The successful candidate will support and collaborate with research groups at the FMI. Time for independent research can be negotiated, based on research experience.

We seek a highly motivated scientist with a track record of several years in computational analysis, such as genomics and proteomics, and preferably with a PhD in molecular biology/biochemistry or a related field. Preferred skills include scripting in R and languages like Perl and familiarity with the common bioinformatics and data analysis tools. Experience with sequence and structure analysis, pathway and gene set analysis tools, Bioconductor, genome browsers, protein function analysis, integrative analysis, statistical data analysis and the development of simple databases with web interfaces is a plus. The successful candidate has strong communication skills and will interact daily with FMI students and postdocs.

As part of the Novartis Research Foundation, the FMI is an international biomedical research centre with 300 members pursuing fundamental research in the areas of Neurobiology, Cell Growth and Signalling, and Epigenetics (www.fmi.ch). The institute is situated in Basel, a city offering an outstanding scientific and cultural environment in the centre of Europe.

Applications, including a detailed CV, names and contact details of three referees and a summary of current and future research interests, should be sent to human.resources@fmi.ch or by post to Human Resources, Friedrich Miescher Institute, Maulbeerstrasse 66, 4058 Basel, Switzerland.

W123353R

LEUKAEMIA RESEARCH
HODGKIN'S | LYMPHOMA | MYELOMA

Scientific Director

£50,000 to £55,000
Holborn, central London

Are you the scientist with the ambition, drive and vision to be our next Scientific Director? Do you have the skills and motivation to manage and develop our research portfolio throughout the UK? Can you progress our mission to achieve the routine cure of all blood cancers and related conditions?

The Scientific Director will be responsible for managing all aspects of the receipt, review and award of applications for funding; identifying new opportunities for funding initiatives; managing the research budget; exploitation of intellectual property; advising the Board of Trustees on all research related matters; organising research meetings and conferences and promoting the research portfolio as part of our fundraising activities.

The ideal person will be a confident manager with a proven track record of excellence and achievement in biomedical research and teaching. Expertise in haematology or a related discipline whilst advantageous is not essential.

This is an outstanding opportunity to take on a fresh challenge and to shape our future research portfolio for the benefit of all patients with a blood cancer.

Visit www.lrf.org.uk/jobs for further information and application details. You must be a non-smoker.

CLOSING DATE: Friday 15th February 2008

Gemma Gillard
ggillard@lrf.org.uk
020 7405 0101
Head Office: Leukaemia Research,
43 Great Ormond Street, London, WC1N 3JJ

Registered charity 216032 (England & Wales) SC037529 (Scotland)

U123861R

GETTING CLOSER TO A CURE THANKS TO YOU

A PROUD TRADITION, AN EXCITING FUTURE

Building on our heritage in surgery, we will enhance human health through endeavour, innovation and collaboration in education, research and service

RCSI

Royal College of Surgeons in Ireland
Coláiste Ríoga na Máinlia in Éirinn

The promotion of Translational Medicine Research and Teaching is a key strategic theme of the Royal College of Surgeons in Ireland (RCSI). Accordingly, we are establishing the following Chair:

Professor of Translational Medicine REF: HR8022

The Chair will head a new centre of excellence for translational medicine and will lead a research programme in one of the RCSI research pillars namely, Cardiovascular Science, Molecular Medicine, Neuroscience, Respiratory and Cancer Cell Biology. The Chair will perform a leadership role in the Clinical Research Centre (www.clinicaltrials-ireland.com/). The appointment will be held within the RCSI Research Institute (www.rcsi.ie/research/) with affiliation to the Department of Medicine. The post includes an outpatient attachment to Beaumont Hospital.

Applicants should hold an MRCPI or equivalent and preferably also a PhD and in addition be eligible for medical registration to practice in Ireland.

The successful candidate will be a skilled communicator with excellent leadership qualities and demonstrate a track record of excellence in biomedical research, with special reference to translational medicine, including expertise both in basic and related clinical research. Experience in co-ordinating national or international research programmes / clinical trials will be an advantage as will experience with collaborating with the pharmaceutical industry.

Applicants should provide explicit evidence for dedication to teaching and curriculum development and demonstrate a record of significant peer-reviewed grant funding and be competitive for programme-type funding awards from grant agencies such as The Welcome Trust, HRB and SFI.

Further details and a copy of the Job Description are available at www.rcsi.ie/careers

Informal enquiries about this post may be made to: Professor Gerry McElvaney, Tel: +353 1 809 3763 Email: gmcelvaney@rcsi.ie or Professor Brian Harvey, Director of Research, RCSI, Tel: +353 1 402 2732 Email: bjpharvey@rcsi.ie

Application Procedure

Interested applicants should submit a cover letter quoting the reference number HR8022, a detailed curriculum vitae and names and addresses of four referees to: Bernard Cahill, Director of Human Resources, RCSI, 121 St. Stephen's Green, Dublin 2. Tel: +353 1 402 2339 Email: recruit@rcsi.ie

The closing date for receipt of applications is: **Friday, 22nd February 2008.**

W123027R

RCSI is an equal opportunities employer www.rcsi.ie

MANCHESTER 1824

The University of Manchester

www.manchester.ac.uk/jobs

Faculty of Life Sciences

Postdoctoral Research Associate

£26,666 - £32,796 p.a. **Ref: LS/016/08**

You will join a research team, led by Dr Christoph Ballestrem, within the Wellcome Trust Centre for Cell-Matrix Research www.manchester.ac.uk/wtccmr The project focuses on how cell interactions with their environment are regulated by proteins localized in cell-extracellular matrix adhesion sites (focal adhesions).

You should have (or expect to hold) a PhD in a relevant subject with practical experience of cell biology, standard molecular biology techniques, and biochemistry (e.g. western blot) and high interest in advanced-microscopy techniques is desirable.

This position is funded by the Wellcome Trust for two years initially, with the possibility of an extension for a third year. You will also be highly encouraged to apply for postdoctoral fellowships.

Application forms and further particulars are available from our website or by contacting +44 (0) 161 275 5777 or annmarie.entwistle@manchester.ac.uk quoting the reference number.

Closing date: 15 February 2008.

The University will actively foster a culture of inclusion and diversity and will seek to achieve true equality of opportunity for all members of its community.

U123914R

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nature methods

Locum Assistant Editor

Nature Methods seeks a Locum Assistant Editor to join their editorial team for a period of six months to cover a maternity leave. The journal publishes high quality papers that represent major methodological developments, likely to be influential in the life sciences. In the tradition of *Nature* journals, this selection relies on a thorough peer review process.

For more information about the journal, see our website (**http://www.nature.com/nmeth**).

Members of the editorial team evaluate manuscripts, oversee the peer review process, commission and edit secondary materials such as Reviews, and write short pieces and editorials for the journal. The new editor will join our team in the NYC office of the larger Nature Publishing Group.

Candidates should have a broad interest in science, excellent communication skills, and a willingness and ability to learn new fields. Applicants should have completed a Ph.D. in any of the areas covered in *Nature Methods*.

To apply, please submit a CV, and a cover letter explaining your interest in the position and your possible start date to Human Resources Department, Nature Publishing Group, e-mail: **admin@natureny.com**

Applications should arrive as soon as possible with a *close date of February 28, 2008.*

NPG is an EOE.

nature publishing group npg

IN123698R

100 Top Hospital expanding in Central Texas

TEXAS A&M INSTITUTE OF REGENERATIVE MEDICINE IN TEMPLE, TX.

Post-doctoral and Faculty Positions

The newly established Texas A & M Institute of Regenerative Medicine is seeking post-doctoral fellows and faculty for research on adult stem/progenitor cells. The Institute is dedicated to research both on the basic biology of stem/progenitor cells and their potentials for therapies of human diseases. It will occupy newly renovated laboratories and a series of core laboratories in an expanding academic medical center. Post-doctoral appointments will be for one year with the opportunity to renew for a second and third year subject to performance. Faculty appointments will be in an appropriate academic department and will range in rank from tenure-track assistant professors to tenured full professors depending on qualifications. Faculty appointments will include institutionally-funded salaries, start-up funds, modern laboratory space, and access to graduate students. Salaries and benefits are competitive. Candidates should have excellent verbal skills and a Ph.D. or M.D. degree from a well recognized university.

Before March 1, 2008, please send curriculum vitae, brief statement of research interests, indication of level of appointment sought, and three letters of recommendation to attention of Darwin J. Prockop, M.D., Ph.D., Director, Texas A&M Institute of Regenerative Medicine at email address: Regenerate@medicine.tamhsc.edu. The Texas A&M Health Science Center is an AA/EO Employer.

NW123456R

SCOTT & WHITE

TEXAS A&M HEALTH SCIENCE CENTER COLLEGE OF MEDICINE

nature REVIEWS

Chief Editor Position

Nature Reviews Genetics - the most highly-cited monthly review journal in genetics and heredity - is seeking to appoint a new Chief Editor with the remit to further develop the journal's coverage and impact. This is a challenging, exciting and rewarding role with overall responsibility for commissioning and editing reviews, managing the editorial team, representing the journal at international conferences and planning special issues and projects.

The ideal candidate will have a PhD and post-doctoral experience in a relevant subject area and a broad interest in genetics and genomics. Although desirable, previous publishing experience is not essential; however, candidates should have genuine enthusiasm for science communication and an understanding of the editorial process. Other important attributes include a dynamic, outgoing personality and good team-working skills. The position includes line-management responsibility so people management experience is also desirable.

The position is based in Nature Publishing Group's modern London offices, and the terms and conditions are highly competitive, reflecting the critical importance and responsibilities of the role. For further information about *Nature Reviews Genetics* please visit http://www.nature.com/nrg. Applicants should send a CV and a brief cover letter explaining their interest in the post, quoting reference number NPG/LON/806 to: Denise Pitter, Personnel Assistant, Macmillan Publishers at londonrecruitment@macmillan.co.uk

Closing Date: Monday 11th February 2008

nature publishing group npg

IN124056R

www.mssm.edu

MOUNT SINAI SCHOOL OF MEDICINE

FACULTY POSITION CANCER CHEMICAL BIOLOGY

The Departments of Structural and Chemical Biology and Oncological Sciences at Mount Sinai School of Medicine invite applications for a tenure-track or a tenured faculty position at the level of Assistant, Associate or Full Professor in the area of Cancer Chemical Biology. We are committed to developing innovative research in mechanisms and processes of cellular signaling and gene regulation and structure based design for novel cancer therapies.

We seek an outstanding candidate with a PhD or MD/PhD degree and demonstrated excellence in synthetic chemistry and chemical biology approaches to the understanding of the molecular basis of human oncology. You will establish a dynamic, independently funded research program and participate in graduate training at the interface of chemistry and cancer biology. You will hold a joint appointment in the two departments in addition to receiving generous start-up funding.

We offer a salary commensurate with experience and a competitive benefits package. Please submit a curriculum vitae, a brief statement of research interests and future plans, copies of 2-3 publications and three letters of reference (sent independently) to: **Chemical Biology Search Committee, Mount Sinai School of Medicine, 1425 Madison Avenue, Box 1677, New York, NY 10029. Email: CSCB.search@mssm.edu.** EOE

NW123768R

FACULTY POSITIONS IN CELL SIGNALING

University of Texas Medical School – Houston

The Department of Integrative Biology and Pharmacology in the University of Texas Medical School at Houston has tenure track faculty openings for researchers with a focus on the cell biology, physiology and pharmacology of cell signaling. Appointments will be at the Assistant Professor level for candidates completing their postdoctoral training, or at the Associate Professor level for candidates with funded, independent research programs. Applicants using innovative *in vitro* or animal model systems, or computational and systems approaches to study cell signaling and/or physiological regulatory mechanisms will be given preference. Responsibilities include the development of a funded, internationally recognized research program that complements existing research activities in the department (**http://ibp.med.uth.tmc.edu**) and participation in medical and graduate education. The Medical School is located within the Texas Medical Center, which includes UT-Houston Health Sciences Center, Baylor College of Medicine and M.D. Anderson Cancer Center. Attractive start-up packages and competitive salaries and benefits will be provided.

Send a curriculum vitae, a description of future research plans and at least three letters of reference to: **Dr. John F. Hancock, Department of Integrative Biology and Pharmacology, University of Texas Medical School** *by email to* **ms.ibp.applicants@uth.tmc.edu** *or by mail to* **Houston, P.O. Box 20708, Houston, TX 77225.** Review of applications will begin immediately and will continue until the positions are filled.

The University of Texas is an Equal Opportunity/ Affirmative Action Employer. M/F/D/V. This is a security sensitive position and thereby subject to Texas Education Code § 51.215. A background check will be required for the final candidate.

NW123703R

Hank Gardner and Marilyn Fiske Chair of Physiology University of Wyoming

The Department of Zoology and Physiology at the University of Wyoming is seeking to fill a full-time, nine-month FACULTY POSITION at the

Associate/Full Professor

level. We are looking for PhD or M.D. candidates conducting innovative research in physiology who are able to integrate research and teaching in organ system physiology. The incumbent will be expected to have an externally funded research program and teach in the department's physiology curriculum which prepares students for further training in physiology and the health sciences. Start up and annual support for the chair is available. Departmental research strengths include comparative physiology, cell biology and physiology, neuroscience and outstanding microscopy and macromolecular facilities.

Interested applicants should send curriculum vitae, statement of research and teaching interests to: **Gardner Physiology Chair Search Committee, Department of Zoology and Physiology, Dept. 3166, 1000 E. University Avenue, Laramie WY 82071.** Fax **307-766-5625.**

For further information contact Dr. Hank Harlow email: **hharlow@uwyo.edu**

Website: **http://uwadmnweb.uwyo.edu/Zoology**

The University of Wyoming is a Carnegie Foundation Research/Doctoral Extensive Institution and is an AA/EEO employer

NW123729R

GEOPHYSICAL LABORATORY
CARNEGIE INSTITUTION OF WASHINGTON

STAFF SCIENTIST

The Geophysical Laboratory of the Carnegie Institution of Washington invites applications for Staff Scientist positions. We seek energetic and creative scientists to develop and carry out world-leading research in fields that complement and extend the current strengths of the Geophysical Laboratory.

The Laboratory emphasises interdisciplinary experimental and theoretical research programs spanning materials physics, chemistry, biology, geoscience, and planetary science. It supports world-class facilities in high-pressure science; synchrotron radiation research; computational and experimental mineral physics and petrology; geochemistry; organic-, stable-isotope, and bio-geochemistry; microbiology and astrobiology.

Applications including CV, list of personal references, and research plans should be submitted to:

Russell J. Hemley, Director
Geophysical Laboratory
Carnegie Institution of Washington
5251 Broad Branch Road
W 20015, USA
or email to
dappleby@ciw.edu

CARNEGIE INSTITUTION FOR SCIENCE

NW123846R

MASSACHUSETTS GENERAL HOSPITAL CANCER CENTER

HARVARD MEDICAL SCHOOL

Lymphoma Research

Massachusetts General Hospital Cancer Center and Harvard Medical School

The Massachusetts General Hospital Cancer Center is seeking to recruit an investigator with a strong research interest in the biology and/or treatment of lymphomas. We seek outstanding individuals with a strong ongoing research program in this area who will be interested in engaging both with physicians at MGH with an interest in lymphoma, as well as with the large community of scientists and physician/scientists at the MGH Cancer Center. Candidates at any stage of their career are encouraged to apply. Applications from women and minority candidates are strongly encouraged.

Candidates should submit a curriculum vitae including a full list of publications and a brief statement of research interests to the address below. Letters of reference may be sought at a later stage in this process.

Lymphoma Search Committee
c/o Carol Ann Hannan, MGH Cancer Center
13th Street, Building 149, Room 7204
Charlestown, MA 02129

Applications must be received by 15th March 2008.

Massachusetts General Hospital and Harvard University uphold a commitment to affirmative action and equal opportunity.

NW123305R

University of Saskatchewan

Assistant Professor

Department of Physiology College of Medicine

We invite applications for a tenure-track position at the level of Assistant Professor.

Candidates must have a Ph.D. or M.D. and post-doctoral experience. She or he must have demonstrated excellence in research in regulatory and integrative Physiology. The successful applicant will teach Physiology to medical students in an integrated and interdepartmental course. He or she is expected to participate actively in the educational activities of the College of Medicine. She or he is expected to develop a strong externally funded research program and to submit an application to the Canadian Foundation of Innovation (CFI). College and University research funds are available as are opportunities for collaboration with various research groups.

The University of Saskatchewan is committed to Employment Equity. Members of designated groups (women, aboriginal people, people with disabilities, and visible minorities) are encouraged to self-identify on their applications. All qualified candidates are encouraged to apply, however, Canadians and permanent residents will be given priority.

Please send curriculum vitae, a brief description of proposed research interests and the names of three references by February 28, 2008 to:

Dr. Wolfgang Walz
Head of Physiology, College of Medicine
107 Wiggins Road, Saskatoon SK S7N 5E5
Canada
Email: wolfgang.walz@usask.ca
Fax: (306)966-4298
Website: http://www.medicine.usask.ca/physiology

NW123589R

UNIVERSIDAD DE LOS ANDES

Faculty Positions

The Department of Chemistry at the Universidad de los Andes in Bogotá D.C., Colombia, invites applicants for

FULL-TIME PROFESSOR

positions and visiting professor in the areas of Biochemistry, Organic Chemistry and Geochemistry.

Applicants should have a Ph.D. degree in the area of interest.

Candidates must be committed to excellence in teaching and research.

Applicants should submit a detailed C.V. and make arrangements to have recommendation letters sent to:
jumoreno@uniandes.edu.co

W123919R

POSTDOCTORAL POSITIONS

Available immediately to study the genetics and cell and molecular biology of aging in yeast (e.g. see Aging Cell [2007] 6, 405).

Send CV and three references to:
S. Michal Jazwinski, PhD, Tulane Center for Aging, Tulane University Health Sciences Center, 1430 Tulane Ave., SL-12, New Orleans, LA 70112

Electronic applications: **(sjazwins@tulane.edu)** will receive prompt attention
AA/EOE

NW123279R

Nagasaki University

Positions Open for the Strategy for Fostering Young Scientists (Assistant Professor)

Nagasaki University in Japan is now actively recruiting three academic researchers as assistant professors for the "Strategy for Fostering Young Scientists." This program has been designed to provide a suitable research environment for young scientists, and to promote their original research by using resources from the "2007 Special Coordination Funds for Promoting Science and Technology" of the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

An assistant professor recruited by this program shall be expected to participate in any of the three major research projects below designed by the University. During a project, the assistant professor will be able to work on his/her independent research supported by research space, facilities, funding, and a respectable salary. If the research achievements are deemed excellent at the end of the determined period, he/she will be promoted to an associate professor following an examination.

- Chronic Pain Research and Control
- Materials Science Based on Nano-Dynamics
- Technology Integration Using Real-time Information Processing

* For detailed application information of each project, please refer to our official website at **http://www.nagasaki-u.ac.jp/wakate/index_e.html**

Scheduled Research Period:
From March 1, 2008 to March 31, 2012

Salary:
Approximately JPY 7,000,000 per year
(Standard Nagasaki University employment rules and regulations will apply.)

Research Fund:
Approximately JPY 6,000,000 per year for the first two years

Application Deadline:
5:00 p.m. on Thursday, February 14, 2008

After passing the preliminary document examination, successful applicants are expected to come to Nagasaki University to attend a seminar in which they will give a lecture and take an oral examination on their current research. The University will cover airfare and transportation fees. In the case of international applicants, the visiting schedule may be arranged with the University.

* Please refer to our official website below for requested documents, qualifications, contact information, and address for submitting application.
http://www.nagasaki-u.ac.jp/wakate/index_e.html

JP123169R

nature

Business Development Executive

Nature is the world's leading weekly scientific journal and is the flagship publication of Nature Publishing Group.

We are now looking for a full-time Business Development Executive to work on Nature special sales both in print and online.

The successful candidate will lead Nature's special sales and business development, including but not restricted to Nature Insights, Nature Outlooks and online initiatives and will be expected to generate and lead new publishing projects both in print and online. General publishing knowledge is desirable whilst a business development/sales background is essential. The position would especially suit those with a business development background who wish to learn more about publishing, and use their sales skills to develop publishing initiatives which generate both editorial and commercial impact.

Candidates should be confident, self starters, able to work independently, and have a proven track record in revenue generation and business development. They should also have a keen interest in scientific communication, and publishing in general. Candidates should have an analytical approach to problem solving and a keen understanding of project management. Strong interpersonal skills and a customer service ethos are essential.

The position will be full-time.

The position is based in our modern London offices.

Please send your CV, a summary of relevant experience, and your current salary, quoting reference number to NPG/LON/812, to Geetika Juneja Personnel Assistant at londonpersonnel@macmillan.co.uk

All candidates must demonstrate the right to live and work in the UK to be considered for the vacancy.

Closing date: 7th February 2008

nature publishing group npg

IN123102R

nature

Managing Editor

Nature is the world's leading weekly scientific journal and is the flagship publication of Nature Publishing Group.

With its authoritative journalism and opinion, a leading position in its science research content, and worldwide influence and engagement, *Nature* stands ready to undertake a period of further investment in both print and online formats. The publisher and the Editor-in-Chief of *Nature* wish to employ a senior manager who will take direct responsibility for the implementation of the publishing programme and for key aspects of publishing and editorial management.

Applicants must have a demonstrable familiarity with the scientific landscape, strong commercial drive, and the ability to manage projects and to achieve demanding goals in a way that stimulates and inspires the colleagues on whom they depend.

The job is based in the London offices of the Nature Publishing Group (NPG), and involves close interactions with colleagues in other parts of Europe, the United States and the Asia-Pacific. The Managing Editor will report to the Editor-in-Chief of *Nature* and to the Managing Director of NPG.

Candidates should have a strong commercial drive, prior editorial experience, preferably in scientific publishing, and some previous experience of the commercial side. They should be comfortable with print and online media and have experience of running projects and managing teams.

The position will be full-time.

The position is based in our modern London offices.

Please send your CV, a summary of relevant experience, and your current salary, quoting reference number to NPG/LON/815, to Geetika Juneja Personnel Assistant at londonpersonnel@macmillan.co.uk

All candidates must demonstrate the right to live and work in the UK to be considered for the vacancy.

Closing Date: 14th February 2008

nature publishing group npg

IN123677R

BESSY

BESSY *operates one of the world's most modern synchrotron radiation sources for VUV and soft X-rays, delivering high quality synchrotron radiation to more than 1300 international users annually. Among other research activities, BESSY operates in collaboration with the Freie Universität Berlin three experimental stations for macromolecular crystallography.*

To strengthen the BESSY-MX team and to take a leading role in the operation and development of our MX-beamlines we are looking for a

Beamline Scientist Ph.D.

Ref. E-MX 08

at the next possible date. The strongly team oriented work will additionally require a close collaboration with the structural biology groups of Freie Universität Berlin and the Max-Delbrück-Center Berlin. Candidates are expected to hold a Ph.D. in physics, chemistry or a comparable qualification and have postdoc experiences in macromolecular crystallography with a convincing research record. Furthermore, a proven expertise in the operation and instrumentation of MX-beamlines is highly desirable.

We are offering a tenure-track position with an initially three-years contract.

Enquiries should be directed to Dr. Christian Jung, (+49 30 6392- 2944), E-mail: christian.jung@bessy.de.

Women are especially encouraged to apply. Handicapped persons will be given preference to other applicants with the same qualification.

E-mail applications will not be accepted. To apply please send a cover letter with the reference code and your complete application documents to:

Berliner Elektronenspeicherring-Gesellschaft
für Synchrotronstrahlung m.b.H. (BESSY)
Personalverwaltung
Albert-Einstein-Str. 15, 12489 Berlin, Germany

W123794R

www.bessy.de

Leibniz Gemeinschaft

nature chemistry

Associate Editors

The Nature Publishing Group is pleased to announce the launch of *Nature Chemistry* in 2009. Following the success of *Nature Materials, Nature Chemical Biology and Nature Physics,* and given the strength of the parent journal *Nature,* we fully expect *Nature Chemistry* to seize the commanding heights of the chemistry-publishing landscape.

Alongside the highest-quality original research, *Nature Chemistry* will cover news, commentary and analysis from and for the chemistry community, as well as striving to develop a voice that chemists care about.

As part of this exciting new publishing venture, we are now seeking three Associate Editors for *Nature Chemistry,* to be based in our London, Boston and Tokyo offices.

Applicants should have a PhD in a chemistry-related discipline, with demonstrable research achievements. Although postdoctoral experience is preferred (not required), emphasis will be placed on broadly trained applicants with a good knowledge of the chemistry community. Key elements of the position include the selection of manuscripts for publication, and commissioning, editing and writing other content for the journal. Candidates who wish to be considered for the role in our Japan office must demonstrate a good understanding of the East Asian research communities (in particular Japan, China and Korea) as well as being fluent in English and preferably an Asian language (Japanese, Chinese or Korean).

These are demanding and extremely stimulating roles, which call for a keen interest in the practice and communication of science. The successful candidates will, therefore, be dynamic, motivated and outgoing, and must possess excellent interpersonal skills. The salary and benefits, will be competitive, reflecting the critical importance and responsibilities of each position.

Applicants should send a CV (including their class of degree and a brief account of their research and other relevant experience), a News & View style piece (no more than 500 words) on a recent paper from the chemical literature, and a brief cover letter explaining their interest in the post, salary expectations, and indicating whether they wish to be considered for a position in London, Boston or Tokyo.

To apply please send your CV and covering letter, quoting reference number **NPG/LON/797** to Denise Pitter at londonrecruitment@macmillan.co.uk

The closing date for applications is Thursday 31st January 2008.

nature publishing group npg

IN121118R

Don't miss the intoxicatingly good job opportunities in *Nature* each week and on naturejobs.com

naturejobs

University of Zurich

The achievement of excellence in cancer research is one of the long-term strategic goals of the University of Zurich. As part of this initiative, the faculties of medicine and science are seeking to fill the position of an

Assistant Professor in Molecular Cancer Research (tenure track)

We are searching for outstanding individuals with a background in life sciences and a track record in the study of biological pathways relevant to cell transformation and cancer. The successful candidate will be expected to establish an independent research group within the **Institute of Molecular Cancer Research** (www.imcr.uzh.ch). The candidate will have access to state-of-the-art research facilities provided by the host Institute and by the Functional Genomics Center Zurich (www.fgcz.ch). There are also excellent opportunities for interactions with other groups of the University of Zurich and the Swiss Federal Institute of Technology (ETH), as well as with the National Centers for Competence in Research (www.snf.ch/en/rep/nat/nat_ccr_pro.asp).

Applications including a detailed curriculum vitae, publications list, short statement of research interests and the names and addresses of three referees should be submitted before February 29th, 2008 to **Prof. Dr. med. Holger Moch, c/o Medical Faculty of the University of Zurich, Pestalozzistrasse 3/5, CH-8091 Zurich, Switzerland.**

The application must include all items requested in the „Instructions for Nominations" of the Medical Faculty of the University of Zurich. The leaflet may be requested from the Dean's office of the Medical Faculty (Fax +41-44-634 10 79) or downloaded from: http://www.med.unizh.ch/FormulareundRichtlinien/Bewerbung.html

The University of Zurich is an equal opportunity employer. Applications from women candidates are particularly encouraged.

W123398R

Universität Bielefeld

The **Faculty of Biology** invites applications for a

Full Professorship (W3) in Biological Cybernetics / Motion Intelligence

beginning October, 1st 2008. Applicants are expected to have an outstanding track record in the field of biological principles of motion intelligence, such as the mechanisms of motor control, the planning of goal-directed behaviour, motor learning, and the control of multiple degrees of freedom. Quantitative experimental research on an appropriate animal model system (preferably an invertebrate system) should be complemented by computational modelling with the perspective of robot applications.

The professorship will play a key role in the recently established **Cluster of Excellence 'Cognitive Interaction Technology' (CITec)** at Bielefeld University. The successful candidate is expected to cooperate intensively with other biologically and especially also technically oriented groups in CITec. The main research focus of CITec is to understand how we can endow technical systems with the necessary cognitive abilities to support humans at a level of semantic interaction offering true flexibility by virtue of adaptivity. To achieve this goal, CITec will unite research groups from computer science, robotics, biology, biomechanics, cognitive psychology and linguistics. CITec is structured around four key areas, intelligent motion, attentive systems, situated communication as well as memory and learning (homepage: http://www.cit-ec.org/).

Candidates should have strong teaching capabilities at the undergraduate and graduate levels and will be expected to teach in the pertinent undergraduate and graduate programmes.

Candidates must have a university and a qualified PhD degree in a pertinent field, strong scientific achievements and an outstanding research record.
We welcome applications from severely handicapped people. We particularly welcome applications from women. Given equal suitability, qualifications and professional achievement women will be given preference, unless particular circumstances pertaining to a male applicant predominate.

Applicants are asked to send their documents no later than **15 March 2008** to the **Dean's Office, Faculty of Biology, Bielefeld University, D-33501 Bielefeld, E-Mail: dekanat.biologie@uni-bielefeld.de.**
The following documents are requested in PDF format: curriculum vitae, publication list, brief statements of research and teaching experience and interests, documentation of successful third-party funding, names and addresses of four references.
(Please note that submitted documents will not be returned.)

W123859R

The Place of Useful Learning

INVESTOR IN PEOPLE

University of Strathclyde

Research Fellow

£26,666

Based in the **Centre for Biophotonics**, this two year Medical Research Scotland funded project "Dissecting the adaptive immune response to cariogenic bacteria in the oral cavity" is in the laboratory of Professor Paul Garside and Dr James Brewer and is in collaboration with Dr Shauna Culshaw at the University of Glasgow Dental Hospital. You should have a PhD and experience in microbiology and/or immunology and bioimaging is desirable.

For an application pack visit http://vacancies.strath.ac.uk or contact Human Resources, University of Strathclyde, Glasgow G1 1XQ, tel 0141 553 4133 quoting Ref JA/R3/2008.

Closing date: 14 February 2008.

U123900RM

We value diversity and welcome applications from all sections of the community.

MDC

MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE BERLIN-BUCH

in the HELMHOLTZ-GEMEINSCHAFT e.V.

The MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE (MDC) Berlin-Buch invites applications for

1 Postdoctoral Position
1 Graduate Student Position

in the group of Prof. Walter Birchmeier, Cancer Biology Department.

Applications are invited from molecular and cellular biologists to join our group. The projects of the group involve the function of signalling molecules in development and cancer (cf. Hülsken et al., Cell 105, 533-545; Soshnikova et al., Genes & Dev. 17, 1963-1968, 2003; Brembeck et al., Genes & Dev. 18, 2225-2230, 2004; Chmielowiec et al., J. Cell Biol. 177, 151-162, 2007; Rosário et al., J. Cell Biol. 178, 503-516, 2007; Klaus et al., Proc. Natl. Acad. Sci. USA 104, 18531-18536).

The positions are funded according to the German TVöD-System.

Applications in writing including full CV and the names of two referees should be sent to

Prof. Dr. W. Birchmeier (wbirch@mdc-berlin.de)
Max Delbrück Center for Molecular Medicine (MDC)
POB 74 02 38, 13092 Berlin, Germany

W123759R

The MDC is a member of the National Helmholtz Research Centers, supported by the Federal Government of Germany and the Land Berlin.

For further information about group and the MDC please visit our World Wide Web sites at:

http://www.mdc-berlin.de & www.mdc-berlin.de/~zelldiff

UKT Universitätsklinikum Tübingen

EBERHARD KARLS UNIVERSITÄT TÜBINGEN

The Medical Faculty of the Eberhard Karls Universität Tübingen invites applications for the position of a

Professor
in Molecular Physiology of the Auditory System

(salary according to Bundesbesoldungsordnung W3)

at the Hospital for Otolaryngology - Tübingen Hearing Research Center to be filled as soon as possible.

The appointee is expected to represent the field of molecular physiology of the auditory system in research and teaching. A strong and competitive research focus on molecular aspects of the peripheral and central auditory system is required. Teaching of students at the Medical Faculty and a contribution to the development of novel teaching programmes is expected.

Prerequisites for the application are a record of excellent research activities equivalent to the German Habilitation and demonstrated teaching skills.

The position is designed as a tenure professorship. If the candidate is entering full professorship for the first time, initially the employment status will be probationary (§ 50 Abs. 1 Landeshochschulgesetz Baden-Württemberg).

The University of Tübingen is indebted to increase the number of female faculty members. Therefore applications from female candidates with suitable qualifications are explicitly encouraged. The University of Tübingen is an equal opportunity employer and handicapped applicants with equal qualifications will be given preference.

Applications including curriculum vitae, copies of relevant documents, a structured list of publications, a detailed list of teaching experience and research grants received, research collaborations, along with a detailed concept of future research should be submitted no later than four weeks after the publication of this advertisement to: **Dekan der Medizinischen Fakultät der Eberhard Karls Universität Tübingen, Professor Dr. Ingo. B. Autenrieth, Geissweg 5, D-72076 Tübingen, Germany**

Please indicate **code number: Zx30**

W123871R

Medicines for Malaria Venture

Associate Director Drug Discovery - Geneva

Medicines for Malaria Venture (MMV) was established in 1999 as a partnership between the public and private sector to discover, develop and deliver new antimalarial drugs at prices affordable to developing countries.

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- Effectively communicating results of Drug Discovery projects to other members of the team and work closely with them to test and improve drug discovery technologies.
- Assuming responsibility for synthetic method development and process optimization for the rapid lead discovery and optimization of clinical drug candidates.
- Understanding the physicochemical properties of new chemical entities and assist in implementing formulations strategies to maximize the bioavailability/exposure in pharmacology and toxicology studies.
- Providing analysis expertise to support decision-making for lead candidate's selections.

Essential qualifications

PhD in chemistry or a related subject with four to five years' experience in project management. Experience of medicinal chemistry and rational drug design is important. Previous experience of infectious disease would be an advantage.

For a detailed job description please visit **www.mmv.org**

Interested applicants should send their complete file before February 29th 2008 to **jobs@mmv.org**

W123631R

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nature materials

Associate Editor

Nature Materials is a prestigious international monthly journal (Impact Factor 19.194) covering all aspects of materials science and technology. We have an exciting opportunity available for a materials scientist or a chemist to join our editorial team as an Associate Editor working on all aspects of the journal.

We are particularly interested in applicants with expertise in physical chemistry and soft matter research but we would welcome applications from outstanding candidates in any area of materials science.

The ideal candidate should have a PhD and preferably postdoctoral experience with a strong research record. The successful candidate will play an important role in determining the representation of their field in the journal, and will work closely with the other editors on all aspects of the editorial process, including manuscript selection, commissioning and editing of Reviews and News & Views, and writing for the journal. A key aspect of the job is liaising with the scientific community through laboratory visits and international conferences.

This is a demanding and intellectually stimulating position. Broad scientific knowledge and training, excellent literary skills and a keen interest in the practice and communication of science are a prerequisite. The successful candidate must, therefore, be dynamic and outgoing and have excellent interpersonal skills. The salary and benefits will be competitive, reflecting the critical importance and responsibilities of this position.

The new editor will join our team in our London office. The *Nature Materials* team is part of a dynamic editorial and publishing environment that also includes *Nature*, *Nature Physics* and *Nature Nanotechnology*.

Applicants should send a CV (including their class of degree and a brief account of their research and other relevant experience), a News & View style piece (600 words or less) on a recent paper from related literature, and a brief cover letter explaining their interest in the post and their salary expectations.

The closing date for applications is Monday 25th February 2007.

To apply please send your CV and covering letter, quoting reference number NPG/LON/823 to Denise Pitter at londonrecruitment@macmillan.co.uk

All candidates must demonstrate the right to live and work in the UK to be considered for the vacancy.

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IN124059R

Nature Clinical Practice Journals

Journal Editor

Ref: NPG/LON/750

Nature Clinical Practice is a major division of Nature Publishing Group, bringing authoritative content to physicians via review titles in eight subject areas (see www.nature.com/clinicalpractice). We now have a vacancy for a full-time Journal Editor on *Nature Clinical Practice Endocrinology & Metabolism.*

Journal Editor is an important and prestigious role, in which the successful candidate will work closely with the journal's internationally renowned Editor-in-Chief and Advisory Board. Key tasks include identifying, commissioning and editing material for publication, developing the content and driving the direction of the journal, and motivating and managing a small editorial team. A keen interest in medical publishing and a proactive approach will be indispensable.

Knowledge of endocrinology and metabolism is preferable but not essential. Previous editorial and line management experience and a postgraduate science degree would be advantageous.

Closing date: Wednesday 6th February 2008

Interviews will take place in the week commencing Wednesday 5th March 2008.

This role will be based in Nature Publishing Group's modern London offices.

Contact Details:
To apply please send a recent CV and covering letter, quoting reference number **NPG/LON/750** to Denise Pitter at londonrecruitment@macmillan.co.uk. All candidates **must** demonstrate the right to live and work in the UK to be considered for the vacancy.

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iCeMS Inauguration Lectures and Ceremony

2008. 02.19
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Kyoto University

iCeMS KYOTO UNIVERSITY
Meso-Control Stem Cells
National WPI Center

iCeMS (Institute for Integrated Cell-Material Sciences) is a newly established international research center at Kyoto University under the World Premier International Center program of the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of the Japanese government. It aims at creating the interdisciplinary fields of meso-control, based on the atomic and molecular interactions occurring in the scale of 5-100 nm, as the cells have designed themselves during evolution, integrated with cell science, with a special emphasis on pluripotent stem cells (for details, visit, http://www.icems.kyoto-u.ac.jp).

10:00-16:30 Lectures
16:30-18:00 Inauguration Celebration Ceremony

Nakatsuji, Norio iCeMS (Director) and Institute for Frontier Medical Sciences, Kyoto University "The scope and aims of the iCeMS, and human embryonic stem cell research"

Huttner, Wieland Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden "The cell biology of neural stem and progenitor cells"

Watt, Fiona Wellcome Trust Centre for Stem Cell Research / School of Biological Sciences, University of Cambridge "Stem cell renewal and differentiation in mammalian epidermis"

Yamanaka, Shinya iCeMS and Institute for Frontier Medical Sciences, Kyoto University "Induction of pluripotency by defined factors"

Takeichi, Masatoshi RIKEN Center for Developmental Biology "Machinery to link cells together"

VijayRaghavan, K. National Centre for Biological Sciences, Bangalore "Actin at the membrane in myoblast fusion during muscle remodelling"

Amatore, Christian Ecole Normale Supérieure "Single Cell Behavior as Investigated by Amperometry at Ultramicroelectrodes"

Rome, Leonard H. California NanoSystems Institute, University of California Los Angels "Engineering of Vault Nanoparticles for Delivery of Therapeutics"

Ryan, John Bionanotechnology IRC / University of Oxford "Real-time molecular imaging of membrane protein structure and function"

Kitagawa, Susumu Deputy Director / iCeMS and Graduate School of Engineering, Kyoto University "Responsive Porous Crystals"

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20 09:00-18:00 Discussion
18:00-20:00 Mixer
21 09:00-17:00 Discussion
17:30-19:30 Posters
22 09:00-13:30 Discussion

Invited Speakers

Davis, Simon Nuffield Dept. of Clinical Medicine and Medical Research Council / Weatherall Institute of Molecular Medicine, University of Oxford

Heuser, John iCeMS (International Affiliate) / Washington University School of Medicine

Kengaku, Mineko (Prospective PI of iCeMS) / RIKEN Brain Institute

Mayor, Satyajit National Centre for Biological Sciences, Bangalore

Nabi, Ivan Robert Department of Cellular and Physiological Sciences / Life Sciences Institute, University of British Columbia

Ritchie, Ken P. Department of Physics, Purdue University

Sasai, Yoshiki RIKEN Center for Developmental Biology

Simons, Kai Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden

Stowell, Michael H. B. Molecular, Cellular, and Developmental Biology, University of Colorado Boulder

Taunton, Jack Department of Cellular and Molecular Pharmacology, University of California, San Francisco

Watt, Fiona Wellcome Trust Centre for Stem Cell Research / School of Biological Sciences, University of Cambridge

Watts, Anthony Biomembrane Structure Unit, Biochemistry Department, University of Oxford

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Killarney, Ireland, May 18–23, 2008
Late Abstract Deadline: Feb. 19, 2008
Early Registration Deadline: March 18, 2008

Malaria: Immunology, Pathogenesis and Vaccine Perspectives
Alpbach, Austria, June 8–13, 2008
Scholarship & Abstract Deadline: Feb. 8, 2008
Late Abstract Deadline: March 11, 2008
Early Registration Deadline: April 8, 2008

Stem Cells, Cancer and Aging
Singapore, Sep. 29 – Oct. 4, 2008

Structural Biology and Activation Mechanisms of Membrane Receptors
St. John's College, Cambridge, UK, Sep. 16–21, 2008

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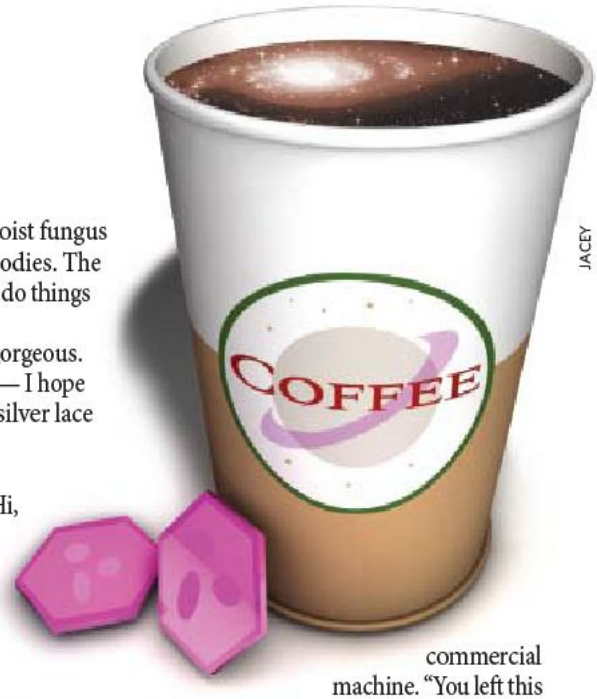
Annie Webber

The customer is always right.



JACEY

Elizabeth Bear

Because I'm an idiot — and because my friend Allan is the coffee-shop owner and my girlfriend Reesa works there — the Monday after Thanksgiving was my first day at a new job.

Total madhouse. Me and Pat foamed milk and drew shots like a flight-line team while Reesa ran the register. It only worked because I'd barista'd at Starbucks and most of the customers were regulars, so they either had their order ready or Reesa already knew it and called it out before they paid. Never underestimate a good cashier.

Allan's has a thing, a frequent customer plan. So Reesa knows the regulars by name.

"Hey, Annie," Reesa said. "Medium cappuccino?"

Annie was petite, ash-blonde hair escaping a seriously awful baby-blue knit cap. She handed Reesa four dollars, then dropped the change into the tip jar.

Cappuccino is nice to make, but it's amazing how badly some people butcher it. I ground beans and drew the espresso. Then I foamed cold milk, feeling the pitcher for heat. When the volume tripled, the temperature was right. The sound of the steam changed pitch. I poured milk over the shot, ladled on foam, and sleeved the cup. "Cinnamon?"

"I'll get my own." She held out her hand. I put the cappuccino in it and set the shaker on the counter.

"You're new here?"

"First day."

"You're good." She sipped the drink. "Annie Webber."

"Zach Jones."

I'd have shaken her hand but there was a coffee in it, and another customer was coming.

That night, Reesa's cat Maggie tried to dig me out of bed by pulling at the comforter. I pushed her off, which woke Reesa. "Wha'?"

Which is all the erudition you can expect at two in the morning.

"Damn cat," I explained.

Reesa pushed her face against my neck. "I only keep her because of the toxoplasmosis."

Running joke. *Toxoplasma* is a parasite that makes rats love cat urine. The parasite continues its life cycle in the cat after the cat eats the rat. According to some show we saw, it affects people too. And the same show had this amazing stop-motion photography of dying bugs, moist fungus fingers uncurling from their bodies. The fungus makes the infected ants do things so it can infect more ants.

The fungus was awful, and gorgeous. One shot showed a moth, dead — I hope dead — on a leaf, netted with silver lace like a bridal veil.

The next morning Reesa said: "Hi, Annie," but a different voice answered: "Hi, Reesa."

I looked up from the steamer nozzle. A big guy, wearing a padded down coat. "Free coffee today?"

Reesa checked the system. "You guys have ten."

He dropped coins in the tip jar. "Medium cappuccino?"

Pat moved to draw it. I gave her a look. "They're all Annie Webber," she said. "By courtesy. Sharing the account."

"Oh."

By the sound, I was scalding the milk. By the time I'd salvaged it, Annie Webber was gone. Reesa waved a pinkish hexagon like a foreign coin. "Zach, what's this?"

I didn't even recognize the metal, let alone the writing.

On day three, the original Annie Webber returned. Day four was number two. On Friday both came, not together. Then half an hour after the second, I served a third. Cappuccino, let me put on my own cinnamon. "Do you guys all drink the same thing?" I asked.

"You guys?" This Annie was a woman, with hazel eyes and crooked nose.

"The Annie Webbers."

She licked foam off her lip. "Nature's perfect food."

I caught Pat's elbow. "How many Annie Webbers are there? How long before I meet them all?"

She counted in her head. "Five come in regular. The blonde and her partners."

"Partners? Like she's poly?"

She shrugged. "I never asked. Maybe they're a cult."

I groped the pinkish coin out. I'd looked it up online, and couldn't find it anywhere.

Saturday, Annie wandered in around ten. The original in the awful toque, scarf snugged under her chin.

I handed her the cup and cinnamon. It takes just seconds to get a good foam with a commercial machine. "You left this Tuesday." I laid the coin down.

"That should have been a quarter. Sorry." She traded for a dollar bill. "Put that in the jar?"

"Annie. It wasn't you here on Tuesday."

"Wasn't it?"

She winked and turned. I yelled "break!" and dove under the counter. Her heels clicked, but this was the smallest Annie. I caught up. Coat flaring, she turned.

"Where do you go?" I asked.

"Excuse me?"

"You. Annie. Where did the coin come from?"

"It was a mistake. I should have looked at the change, but I was out of you… money."

"So you use the free coffees when you've just come back? When you don't have any, what, local money?"

She stared. "I've been coming to that shop since it opened. You're the first to ask."

"You go other places."

"Other … places?"

"Other dimensions."

"You read a lot of science fiction, Zach?"

"You're what, kind of multiple bodies, one mind?"

"*Star Trek*," she said.

"Am I wrong? Why us?" I wondered if I sounded as jealous as I felt.

"Best coffee in the Universe." She kissed me on the mouth, with tongue.

I woke itching. My tongue, my hands. The soles of my feet. When I stumbled to the kitchen, Reesa gave me scrambled eggs, but all I wanted was coffee. Coffee and milk and cinnamon. "Zach?" she asked. I had to bite my lip not to correct her.

That's not my name.

I have to go.

I think I've met all of Annie Webber. ■

Elizabeth Bear won the 2005 John W. Campbell Award for best new writer.

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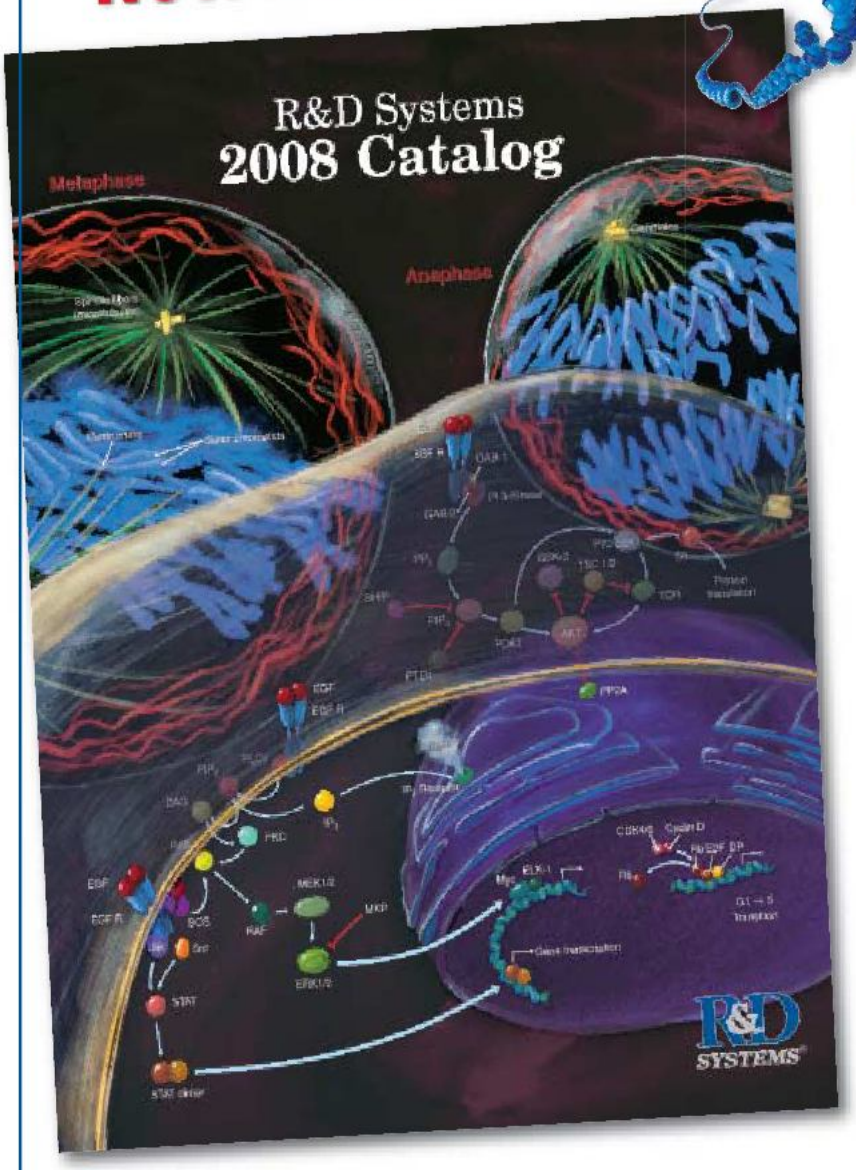

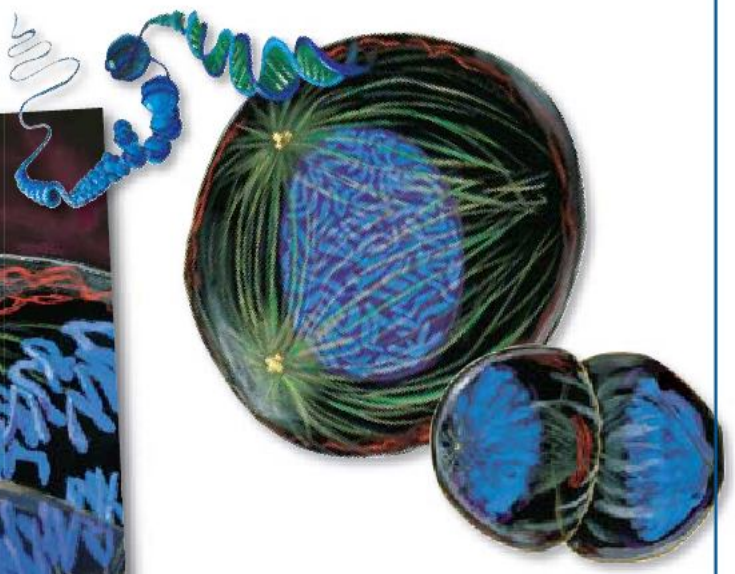

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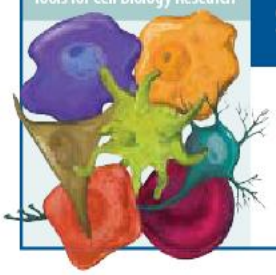

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